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This report discusses fundamental and technical considerations relating to laboratory studies of the fate and effects of organics and metals in biological wastewater treatment processes.

The relationship between analytical control tests and biodegradation test methods are discussed.

Various type laboratory apparatus for both continuous flow and static biodegradation methods are described.

The results of studies conducted by the Wastewater Research Division over a twenty year period are summarized.

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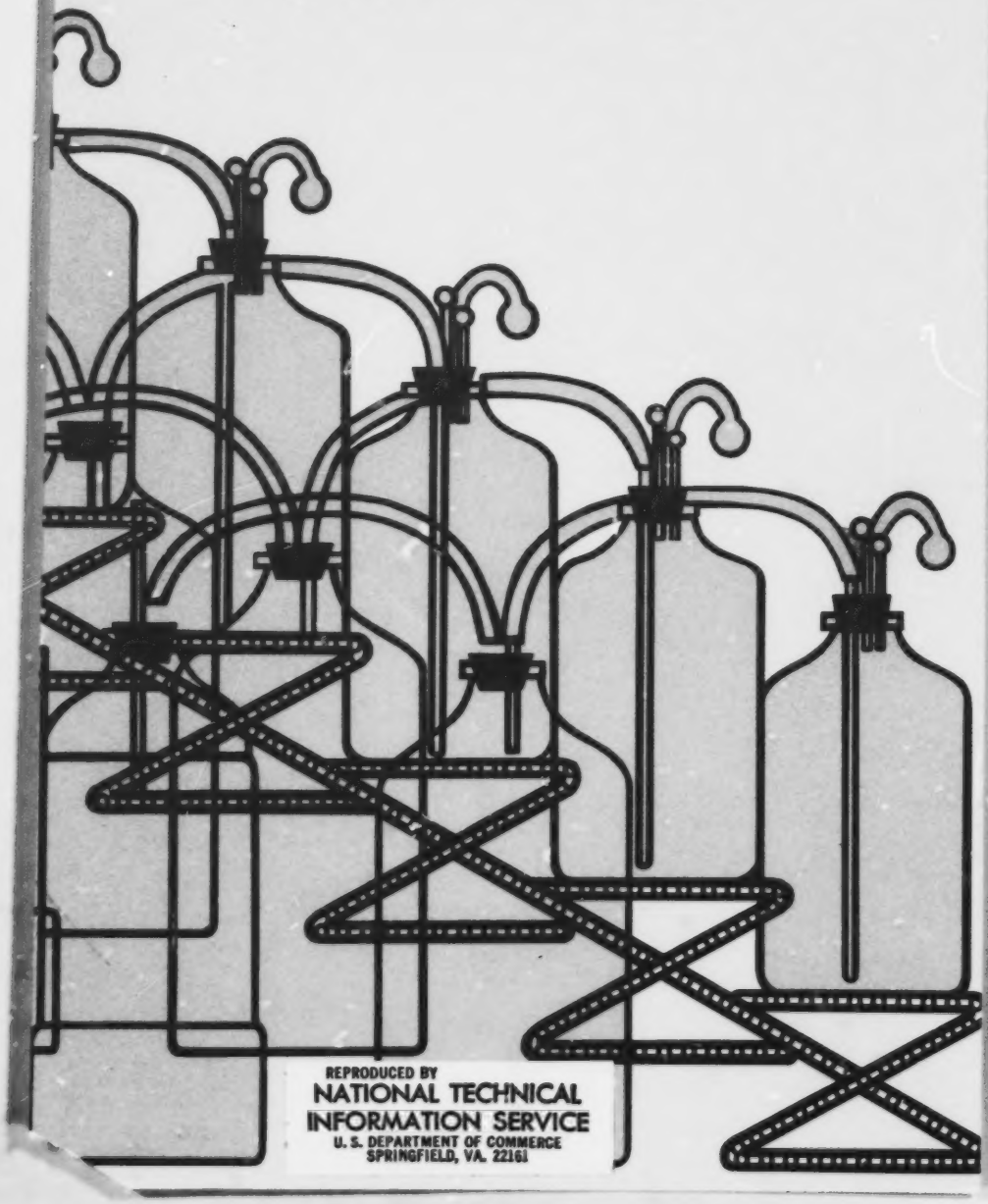

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Biodegradation and Treatability of Specific Pollutants

by
Edwin F. Barth and Robert L. Bunch
Wastewater Research Division

MUNICIPAL ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

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FOREWORD

The Environmental Protection Agency was created because of increasing public and governmental concern about the dangers of pollution to the health and welfare of the American people. The complexity of the environment and the interplay between its components requires a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solving and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment, and management of wastewater and solid and hazardous waste pollutant discharges from municipal and community sources. We are committed to the preservation and treatment of public drinking water supplies, and to minimizing the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of MERL research and, as such, is a most vital communications link between the researcher and the user community.

This report describes biodegradation and treatability studies that have been conducted over a period of 20 years by the Wastewater Research Division.

Francis T. Mayo
Director
Municipal Environmental Research
Laboratory

ABSTRACT

This report discusses fundamental and technical considerations relating to laboratory studies of the fate and effects of organics and metals in biological wastewater treatment processes.

The relationships between analytical control tests and biodegradation test methods are discussed.

Various laboratory apparatuses for both continuous flow and static biodegradation methods are described.

Both aerobic and anaerobic test results are included in the report.

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INTRODUCTION

This compilation of biodegradation and treatability studies is an overview of work conducted by the staff of EPA's Municipal Environmental Research Laboratory over the last twenty years.

The report is not intended to be detailed, but rather discusses approaches, considerations, and techniques evaluated during laboratory studies of specific pollutants.

Several protocols are presented with a summary of the type of results obtained for each. There is no intention at this time of selecting one procedure as the best method for all environmental decisions.

We intend that the report serve as a review and summary of past investigations and provide the user with a framework for deciding on directions to pursue when undertaking similar studies.

John J. Convery
Director
Wastewater Research Division
Municipal Environmental Research
Laboratory

Section 1. Biodegradation of Organic Compounds

FUNDAMENTAL CONSIDERATIONS

Biodegradation, as a theoretical concept, refers to the process by which an organic compound is converted to carbon dioxide, water, and other inorganic constituents by the action of living organisms. In practice, several qualifying terms are used to modify this concept.

The operational definition of biodegradation can be divided into three categories:

Ultimate Degradation: Conversion of the parent compound to carbon dioxide, water, and inorganic compounds (if elements other than carbon, hydrogen, and oxygen are present in the parent compound).

Primary Degradation: A change in the parent compound such that it no longer responds to the analytical measurement used for detection.

Acceptable Degradation: Conversion of the parent compound to the extent that undesirable properties are no longer manifest.

Because of the differences in duration, test procedures used, endpoint criteria, and analytical methods selected, the demarcation between categories is not clear-cut. Futher complicating this situation is the fact that during biodegradation a certain amount of the constituents of the parent compound will be assimilated into the active biomass. Thus, it is impossible to do mass balances for biodegradation studies on a routine basis.

Most of the published work on biodegradation deals with aerobic systems. However, anaerobic degradation can be an important environmental consideration and procedures for measurement of this process are a necessary part of a fully developed set of procedures for observing biodegradation. This is an area that requires considerable developmental work.

The main consideration in biodegradability is whether or not an organism exists that can break down, metabolize, or oxidize the parent compound, either on first exposure to it or after a suitable acclimation period. This acclimation period is important and should be accounted for in all biodegradation studies concerned

with environmental acceptability. Acclimation can allow organisms to produce an induced enzyme in response to the presence of the unfamiliar substrate; or provide time for population shifts to favor an increase in organisms utilizing the specific substrate.

An academic distinction can be made depending on whether a compound is degraded in a biological system where it is the sole source of carbon; or whether degradation takes place in the presence of multiple carbon sources (co-metabolism). From a practical point of view, systems employing co-metabolism are more similar to environmental situations and yield more realistic estimates of biodegradation. Co-metabolism is also important to the treatment of diverse industrial wastes, which are produced when the manufacturer uses a variety of starting materials and alternative reaction pathways. If biological treatment is one of the possible means of disposing of the unusable or unsalable wastes from an operation, the biodegradability of these mixed wastes may be an important factor in the economics of process selection.

The above discussion did not include the factors of time and extent of degradation. In real world situations, the extent of degradation within a fixed time limit is a very important consideration in any working definition of biodegradability. In principle, laboratory testing should show the biodegradability of the material tested as it would occur in the environment or in the wastewater treatment plant. Obviously, most substances will eventually be stabilized by microbial action, given enough time and the right conditions. We must, therefore, consider the biodegradation process as a rate process. For practical purposes related to wastewater treatment, the substance under consideration should degrade at a rate equal to or greater than the degradation of the usual constituents of domestic waste, after the organisms have become acclimated to the substance. A control test utilizing a compound with known biodegradation characteristics, such as linear alkylbenzene sulfonate, should be included in each experiment.

Common experience has shown that if a specific assay is used to monitor the extent of biodegradation, 100 percent degradation is seldom achieved. This is due to the fact that the organisms cannot derive energy by oxidizing compounds at very low concentrations.

TECHNICAL CONSIDERATIONS FOR MICROBIAL GROWTH

The proper conditions must be maintained before any compound can be biodegraded. Microorganisms cannot grow, and hence organic compounds cannot be degraded, unless a number of conditions are fulfilled. All necessary nutrients and growth factors

must be present in the appropriate chemical and physical forms. Solutions are more readily degraded than suspensions. An adequate supply of water and sufficient hydrogen acceptors such as molecular oxygen, nitrate, or carbon dioxide must be present. Because many diverse species of microorganisms are involved with environmental degradation, their growth can occur over a wide range of temperature and pH values; however, for any one specific case there may be optimal values. In general, the factors listed below should be considered in aerobic testing.

pH

The culture medium should be buffered to maintain the pH of the system between 6.5 and 8.5. If the medium contains nitrogenous components and nitrification occurs, alkalinity can be depleted. During the conversion of one part of ammonium nitrogen to nitrate nitrogen, 7.1 parts of calcium carbonate ($CaCO_3$) alkalinity will be destroyed. Sodium bicarbonate is an excellent buffering reagent for pH control.

Dissolved Oxygen

According to the culture method selected, oxygen can be supplied by shaking, diffusion from the atmosphere, or aeration with compressed air. The concentration of dissolved oxygen in the culture medium should not be allowed to fall below about 1 mg/l.

Nutrient Balance

The proximate composition of protoplasm is $C_{106}H_{180}O_{45}N_{16}P_1$, therefore, to ensure that synthesis of new cellular matter is not hindered, the culture medium should have these elements available. In the process of degrading organics, carbon and hydrogen are supplied by the parent compound and oxygen by substrates such as water and air; however, nitrogen and phosphorus must be externally supplemented. For each 1 mg/l of parent compound *carbon* added to the culture medium, at least 0.2 mg/l of ammonium nitrogen and 0.02 mg/l of phosphorus should be provided. Reagent grade phosphoric acid and ammonium hydroxide can be used to supply these two elements.

Trace elements such as sulfur (S), potassium (K), calcium (Ca), and magnesium (Mg) are required in such small amounts that in all but the most unusual cases these will be present in sufficient amounts as contaminants in the carriage water, chemicals used for medium preparation, or inoculum. Preformed growth factors such as thiamine or biotin are usually provided by the addition

of yeast extract or domestic wastewater, depending on the medium selected.

Temperature

Initial screening tests for biodegradation should be conducted at 20°C ± 5°C. This allows experiments to be conducted at ambient laboratory temperatures and is a reasonable temperature for environmental assessment. More information at 30°C and 5°C can be developed if the situation warrants such detail.

Inoculum

Microorganisms to be used as the initial inoculum can be obtained from a wide variety of sources, depending upon the information desired, the culture medium used, and the choice of culture method. For assessment of environmental biodegradation, natural inocula rather than pure culture systems are preferred.

Natural inocula can be obtained from ambient air, forest or garden soil, activated sludge or trickling filter biomass, natural waters, settled wastewater or final effluent from biological processes.

Toxicity

Controls must be included in biodegradation tests to ensure that viable inoculum was used and that absence of degradation is not the result of the initial toxicity of the compound.

If all the culture conditions cited above are controlled, a known degradable compound, which is included as a control in a test system, should show the expected degree of degradation. If not, either the inoculum or the medium must be the source of trouble.

Since co-metabolism studies are recommended for environmental degradation tests, the medium should contain readily degradable materials from yeast extract or wastewater. Therefore, slight microbial growth should be noted even in systems containing non-degradable test compounds. If this growth is absent, it is an indication the test compound is toxic. In that case, the test material should be evaluated at lower concentrations and serial transfers should be performed to accommodate acclimation of the microorganisms.

Medium Selection

Four types of media are commonly used:
Natural waters,

Mineral salts plus yeast extract,
Mineral salts plus yeast extract and supplemental organic materials, and
Settled domestic wastewater.

The first two are used mainly for determination of biodegradation in screening tests. Because of their low organic content, oxygen transfer problems are minimized and cell synthesis is minimal. This latter point is important since the low cell yield precludes concern with sorption of the test compounds on a massive surface. These media are usually employed in batch tests.

The last two media are used for treatability studies. The higher organic content supports luxuriant cell growth. Factors such as sorption to cell mass and varying sludge age can be studied with these media. These media can be utilized for either batch or continuous flow systems.

ANALYTICAL CONTROL FOR BIOLOGICAL TREATABILITY STUDIES

As in any chemical reaction, the process of biodegradation can be followed by observing the disappearance of the reactants (organic compounds and oxygen) or the appearance of products (cell matter, carbon dioxide, and inorganic ions). The disappearance of some specific organic compounds cannot, in many cases, be proof that the compounds have been completely degraded. Most chemical or physical tests depend on a particular functional group of the organic compound. For example, in testing for a surfactant of the alkylbenzene sulfonate type, several methods of assay could be used. If the method were based on detection of the benzene ring, then the benzene ring would have to be degraded for it not to be detected. However, if the method were based on the formation of sulfate, then the molecule would have to be so disintegrated that the sulfonate group would split off the compound.

Specific Analyses

Almost any specific physical or chemical assay can be applied to treatability studies providing the medium or metabolites do not interfere. In many cases preliminary clean-up or concentration steps may be necessary before the final analysis.

The possible assays cover the whole range of analytical tools such as colorimetry, optical rotation, radionuclides, ultraviolet light and infrared light absorption, liquid and gas chromatography, and mass spectrograph.

In order to gauge ultimate degradation as compared with primary degradation, it is necessary to use some type of analytical test

that measures organic matter in conjunction with specific assay. It a low concentration of total organics is measured after degradation, it indicates that partially degraded end-products have not accumulated.

Non-Specific Analyses

For compounds or unknown wastes for which a specific analysis of sufficient sensitivity and applicability in the biological system is not available, a non-specific analysis must be used. The most commonly used methods have been the oxygen utilization or biochemical oxygen demand (BOD), the chemical oxygen demand (COD), and the total organic carbon (TOC). Unlike the methods that depend on the observation of the disappearance of some specific organic compound, these analyses are based on the disappearance of oxygen and organic matter as the degradation proceeds, which is a general function of aerobic systems.

Of the three methods, only the carbon determination (TOC) is a definitive measurement. The BOD and COD tests are empirical tests that give results in terms of the amount of oxygen utilized by matter under a prescribed analytical procedure. Because they do not indicate the quantity of any particular constituent, it is essential that they be conducted according to a standard method.

The BOD Test --

The BOD of a sample is the weight of oxygen consumed by a varying quantity of bacteria in contact with the sample at a specified temperature during a stated time period. The test does not directly measure the amount of organic material present. Phrases such as "BOD is rapidly removed" and "the conversion of BOD to cellular material" should not be used since that type of thinking can lead to incorrect assumptions about the amount of oxygen required to stabilize organic matter under aerobic conditions.

The test does not differentiate matter that is biologically oxidized, from matter that is first synthesized into protoplasm and then oxidized during the endogenous respiration of cellular material. The BOD of a waste will always be less than the theoretical ultimate BOD because the cellular material is relatively resistant to microbial oxidation. Whether or not the BOD includes the oxygen demand used in nitrification will depend on the sample, inoculum, duration of the test, and addition of a nitrification suppressor.

The main limitation of the BOD test is the inapplicability of test results to predicting what will take place when the waste is actually oxidized in a different environment. The BOD test is a measure of avidity for oxygen under specified conditions. It does not necessarily follow that these same conditions exist in a waste treatment plant or a stream. In spite of its imperfections, the biochemical oxygen demand test is useful in measuring easily oxidized wastes in samples requiring more than 3 mg/l of oxygen.

The COD Test --

The chemical oxygen demand is the amount of oxygen consumed by a compound when it is digested with chromic and sulfuric acids. With the proper catalyst, all but a few organic compounds are oxidized to carbon dioxide and water under the conditions of the standard test. The COD test does not measure the oxygen required to oxidize ammonia or organic nitrogen.

The main limitation of the test is that the chemical oxidant reacts with both stable and unstable organic matter. The test does not differentiate between inert and biologically oxidizable matter; neither does it provide any indication of the rate at which the biologically active material would be stabilized under the conditions of waste treatment or in streams.

The test has the advantages of requiring only a short time for the determination and not being affected by toxic wastes. Used in conjunction with the BOD test, it will give an indication of the characteristics of wastes. For example, if the samples contain substances that are difficult for microorganisms to oxidize, the COD value will be much greater than the BOD value.

This procedure is used principally for samples that have a COD value above 10 mg/l. It can also be used on samples containing less than this amount to indicate an order of magnitude of organic content.

The TOC Test --

The most commonly used total organic carbon method depends on the rapid combustion of a micro-sample in a stream of oxygen in a heated tube. The carbon dioxide produced is measured with an infrared analyzer at a wavelength specific for carbon dioxide. This method was designed primarily for analysis of true solutions. Unless the particles in samples are reduced to about 50 microns by blending, the needle used for injection will act as a filter. The usable sample size is 20 μl, so some difficulties may arise in obtaining a representative sample. For this reason, in biodegradation studies the dissolved organic carbon is usually determined. That is, the

analysis is run on a filtered sample. The TOC apparatus is sentitive to about 1 mg/l total carbon, but for quantitative determinations it is desirable to have samples in the 2–3 mg/l range. In order to measure only organic carbon, the inorganic carbon must first be removed from the sample. In samples having a very small amount of organic carbon, it is essential to eliminate completely any inorganic carbon from the sample by first acidifying and then purging with nitrogen. Some of the newer instruments permit the separate determination of inorganic carbon. In that case, the decarbonation step may be omitted as an external procedure.

The advantages of the method are that a definite substance is measured in a very short time, and the results may be interpreted in relation to the thoeretical ultimate oxygen demand. The disadvantage or limitation of the method is that all organic matter is oxidized, which may or may not be the case in biological treatment systems or streams. Many things can be oxidized at high temperatures that would not be oxidized in a stream within a reasonable time. For example, activated carbon is usually not considered to exert a dissolved oxygen demand on a stream, yet it can be oxidized at high temperatures.

BIODEGRADATION TEST METHODS

Over the years considerable research has been done on evaluating microbial breakdown of organic compounds. Techniques have ranged from Warburg respirometry used by microbiologists, to BOD determinations and laboratory activated sludge units used by environmental engineers.

The important features and limitations of various methods of testing biodegradability are discussed below.

River Die-Away --

The river die-away method is very simple and has been used by many investigators. The compound to be tested is added to a sample of river water at ambient room temperature. Periodically, the solution is analyzed for the test compound to establish a die-away or disappearance curve. The curve usually shows a lag period of no degradation for several days and then a rapid removal. The advantage of the river die-away test is that it requires very little equipment and yet can indicate the rate of degradability of the test material. However, the rate is only relative. If, after the disappearance of the compound, an additional amount is added, it will disappear in less time. This can be repeated several times, resulting in a die-away curve with a saw-toothed appearance (Fig. 1).

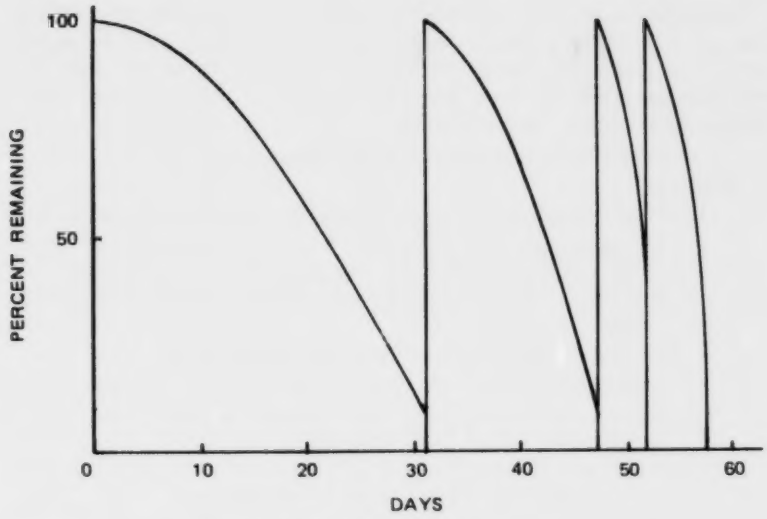


Figure 1. River die-away pattern.

A modification that can be used to reduce the lag time and to speed the process is to add a small amount of activated sludge to the river water.

The disadvantage of the test is that all river waters are not the same. Some have more microorganisms than others, and the pH and mineral constituents vary. To be able to use this procedure, it is necessary to be able to analyze for the compound or some constituent of the molecule that is a likely degradation product. The method of assay will detect the original compound, but perhaps not a slight modification of the compound. Thus, intermediate products may go undetected. In spite of the inherent drawbacks of the river die-away test, it is useful in obtaining preliminary information on the ease of biodegradation. A large number of compounds can be screened in a relatively short time.

Warburg Respirometer --

The disappearance of oxygen as degradation proceeds is a general characteristic of aerobic biological systems. The Warburg respirometer is one of the many apparatuses that permit oxygen uptake rates to be determined for a biological system. The Warburg respirometer is popular because it is commercially available, compact, and permits a number of samples to be run simultaneously.

There are a number of variables that can affect the results, but the most important factor is the type and quantity of the microorganisms used. The seed (inoculum) used should be one that has become adapted to the test compound or has at least been subjected to an adaptation period.

Some of the advantages and disadvantages are:

Advantages:

1. Direct measurement of biological oxidation is possible.
2. This approach can be used for all compounds without using a specific analytical test procedure. This is a decided advantage in cases where no reliable analytical method exists.
3. The results are available within a reasonable time.
4. Oxygen consumption, radioactive-carbon dioxide evaluation, and respiration assimilation balances can serve as bases for measuring biodegradability.
5. Continuous observation of multiple samples over periods of a few hours or several days is possible.
6. Lag periods and rate changes are readily determined.

Disadvantages:

1. Equipment is fairly expensive and requires a skilled technician.
2. The small sample size makes it difficult to obtain representative samples of wastewater and industrial wastes.
3. All organic matter added to the system is subject to oxidation. Therefore, pure organic compounds are needed for fundamental studies unless a specific assay is included in the study.
4. With extended runs, nitrification can occur, giving erroneous high results of oxygen uptake. Each part of ammonium nitrogen converted to nitrate will consume 4.5 parts of oxygen.
5. Carbon dioxide evolved during respiration must be absorbed from the gas phase. It has been reported that complete absence of carbon dioxide can inhibit assimilation of organic matter by bacteria.
6. Oxygen uptake results are difficult to interpret quantitatively unless supplemented by other analyses.

Biochemical Oxygen Demand Test --

The biochemical oxygen demand test (BOD) is one of the older methods for evaluating the biodegradability of substances. It was first published as a standard by the British Royal Commission for Sewage Disposal in 1912. For many years nothing better was available for estimating the strength and controlling the treatment

of wastewater. The sample is stored in a filled bottle for five days in the dark at 20°C, then the amount of dissolved oxygen utilized is measured. Instead of a single final value, a curve showing oxygen uptake as a function of time can be developed if replicates of the original sample are analyzed at different times.

With the development of reliable oxygen-sensing probes, it is now possible to determine the oxygen content of a single sample, at intervals or continuously, over the entire test period. Another modification of the test apparatus is to determine the oxygen uptake electronically based on the electrical current needed to generate the oxygen required to maintain the pressure at equilibrium in the closed flask.

The BOD test has numerous limitations relating to the applicability of the test results to predicting what will take place in a waste treatment plant or a stream. Only the limitations that are pertinent to the utilization of the method for determining biodegradability will be discussed.

The advantages and disadvantages of the BOD test procedure are:

Advantages:

1. The apparatus and testing procedure are simple. The test does not require a highly trained technician.
2. Extensive BOD data are available on compounds with known waste treatability.
3. The small number of bacterial cells used and the organic-free BOD dilution water minimize interferences with specific chemical analyses either for the parent compound or intermediate breakdown products.
4. Carbon dioxide is present throughout the test period and does not become limiting as is possible in the Warburg procedure.
5. Problems of aeration and oxygen transfer are not encountered if suitable initial concentrations are selected, because all the oxygen required is present at the beginning of the test.

Disadvantages:

1. Only a small amount of sample can be used because of the limited solubility of oxygen in water.
2. The testing period is long. However, intermediate results can be obtained in less time if oxygen depletion is measured periodically.
3. As with the Warburg procedure, nitrification and extraneous organic matter can lead to erroneous conclusions.
4. The interpretation of the results has the same limitations as other respirometer methods in that the results are not directly convertible to a percentage of the compound degraded.

Flask Test Method --

Except for the activated sludge method, this is the most widely used of all the laboratory procedures. There are many versions of the technique, but most use a chemically defined medium. The flasks may be aerated by continuous shaking on a mechanical shaker, agitated with magnetic stirrers, aerated through a ceramic filter, stirred with a paddle stirrer, or merely left quiescent. The test mixture can be analyzed each day or at the end of a specified time. A variety of inocula can be selected for this procedure. If degradation is not evident or toxicity is suspected, the test compound should be run at a lower concentration. Parallel runs with known degradable compounds are advisable to confirm that the conditions were correct. It is suggested that a compound of similar structure and class serve as a reference standard.

The popularity of the flask test method is exemplified by the fact that five leading countries (Austria, Canada, Germany,Sweden, and England) have adopted some version of it as their screening or presumptive test method for determining the biodegradability of anionic detergents.

Some of the advantages and disadvantages of the flask test method are:

Advantages:

1. The method does not require elaborate or specialized laboratory equipment.
2. Reproducibility is generally good.
3. Sample size is not severely limited.
4. The test compound does not have to be pure.
5. Metabolic products can be identified without too much interference if mineral salts are used and if the test compound is the major carbon source.

Disadvantages:

1. The method requires a separate adapting process if adaptation is not made a part of the test procedure.
2. Shaking on a mechanical shaker limits the number of samples that can be run. If a static procedure is used, sample concentration is limited owing to poor oxygen transfer.
3. The test does not simulate any waste treatment process.
4. A chemical or physical analytical test method must be available for each class of compounds tested.
5. Simple analytical procedures generally do not measure partial degradation residues.

Activated Sludge Method --

The activated sludge method is one of the most important wastewater treatment processes. Laboratory scale models of the process have been used for many years to study the treatability of various municipal and industrial wastes. It was natural that the method should become a major tool in biodegradability and treatability testing.

The laboratory methods based on the activated sludge principle vary in feed, residence time, and whether the feed is continuous or batch operation. The continuous activated sludge system is preferred because it corresponds more nearly to actual conditions. A system that approximates the operation of a plug flow aeration tank is known as the fill-and-draw or semicontinuous process. In this method, the inoculum, the test material, and the feed are placed in a vessel and aerated. The feed is usually a chemically defined medium with organic supplements. The mixture is aerated, usually for 23 hours. The air supply is then turned off and the mixture allowed to settle (see Fig. 2). A portion of the supernatant is removed and replaced with new feed and the test compound. This sequence is then repeated for the duration of the test. If the test lasts a long time, it is necessary to waste some activated sludge periodically in order to maintain a reasonable level of biomass. As in all aerobic biological processes, there is a net increase in bacterial cells.

Although each cycle in the semicontinuous operation constitutes a batch run, the method closely approaches plug flow conditions in a conventional activated sludge plant. The aeration basin of the activated sludge process is usually long and narrow. The recycled sludge and the wastewater enter at one end of the aeration tank. Ideally, the mixture moves as a plug along the entire length of the tank. The organisms are subjected to all phases of the growth curve from the log phase to the endogenous stage. That is, at the head end of the tank, the organisms have an ample supply of food. It is in this part of the tank that the organisms grow most rapidly, and consequently the rate of removal of organic matter is the greatest. As the plug moves along the tank, the food becomes depleted, and if the retention period is correct, the bacteria will start to enter the endogenous phase near the end of the aeration basin.

Various designs for continuous flow activated sludge studies have been reported in the literature. Figure 3 shows a continuous flow, complete mix reactor of 300 ml capacity developed by Monsanto Chemical Co. Figure 4 shows a 6 liter complete mix, continuous flow unit developed by the U.S. Public Health Service.

AERATION PERIOD (A).

SETTLING PERIOD (B).

Figure 2. Four liter fill-and-draw reactors with two liters of test mixture, in both the aeration period (A), and the settling period (B).

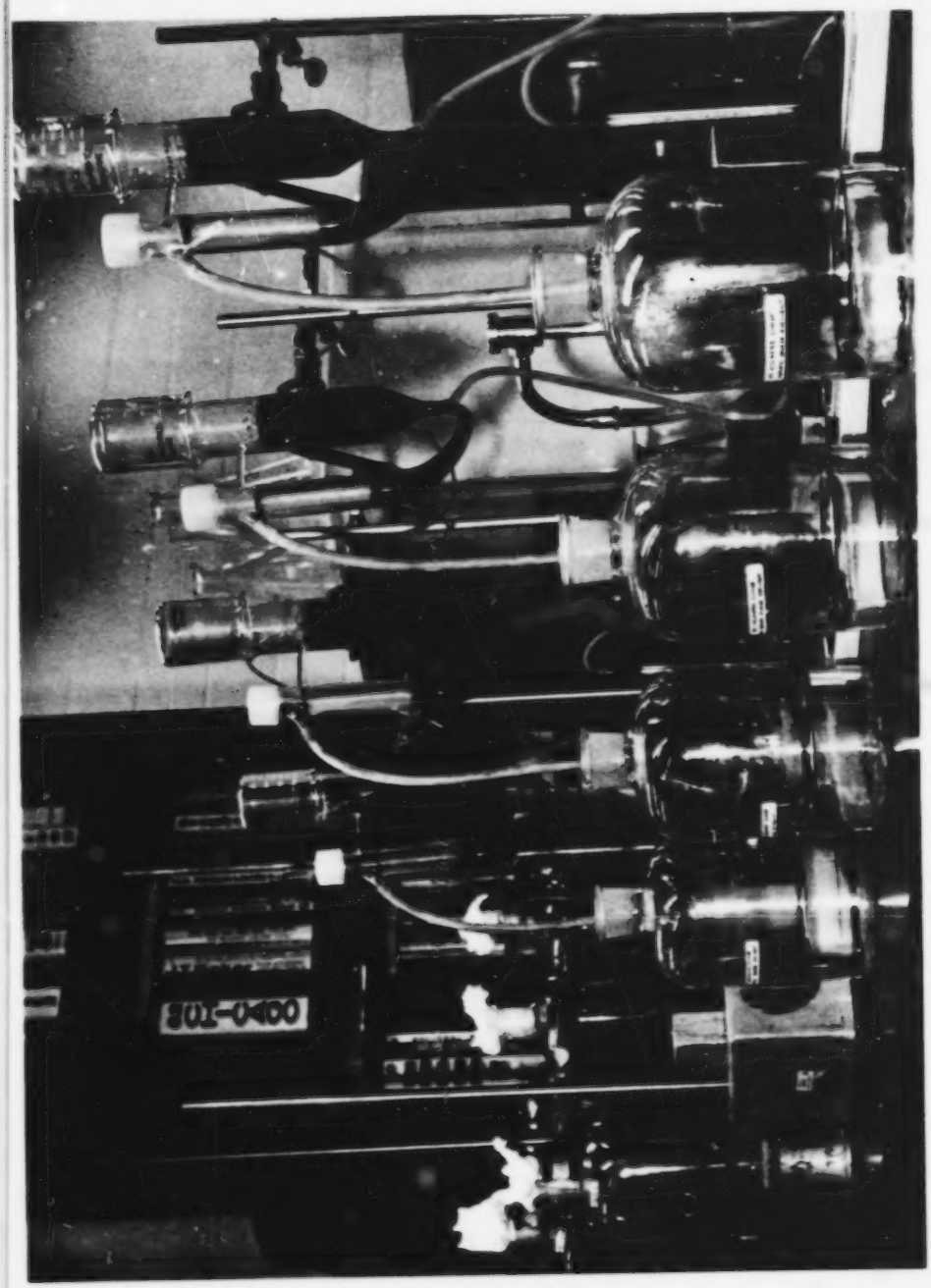
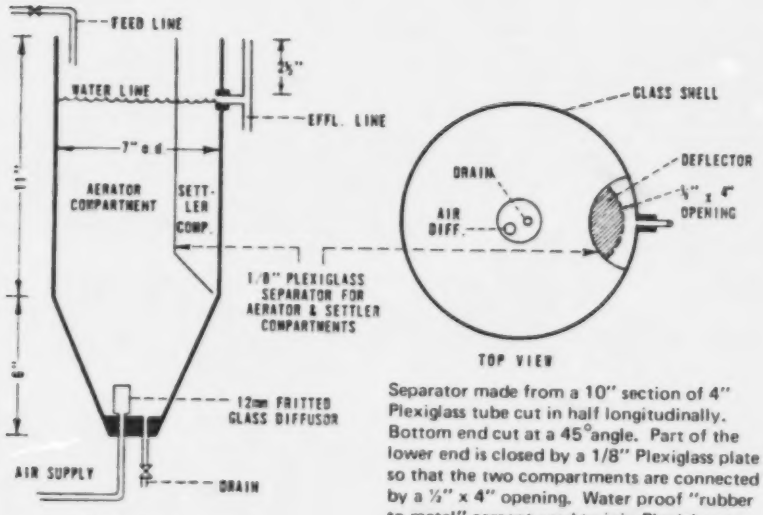


Figure 3. Continuous flow 300 ml reactor.

Figure 19 shows a 750 liter per day continuous flow, plug flow reactor.

Any material used for constructing equipment for biodegradation or treatability studies on very low concentrations of test compounds should be evaluated for sorption of the test compounds on surfaces.

Small reactors of only several hundred milliliters can be used to establish the biological characteristics of a test system, such as degradation or sorption on the sludge surface. The larger units of several liters capacity are useful to collect additional information on the physical characteristics of the system, such as sludge settleability. Finally, the large continuous flow pilot plants of several hundred liters per day through-put capacity are necessary to collect complete treatability and engineering design data. The large pilot units can be used to track disproportionation of a compound through the various unit processes and give guidance on settler overflow rates, sludge production, aeration requirements and detention time for the unit processes.



7" o.d. Pyrex Shell 3/8" wall drawn to a cone. Corning Glass Works Spl. App. Div. Reference No. D459051 - T140

Separator made from a 10" section of 4" Plexiglass tube cut in half longitudinally. Bottom end cut at a 45° angle. Part of the lower end is closed by a 1/8" Plexiglass plate so that the two compartments are connected by a ½" x 4" opening. Water proof "rubber to metal" cement used to join Plexiglass to glass.

Figure 4. Model activated sludge unit.

The test apparatus selected should not be so small that sampling would disturb the system. For biodegradability testing it is not necessary to maintain a large unit. Small units are much more desirable because of space, cost, and labor of servicing several large units. In scaling down to very small units, there is some difficulty in designing settling chambers. In most units the settling chambers are an integral part of the aeration tank rather than being separate units. Most of the laboratory units are the completely mixed type rather than the plug flow type.

A list of some of the advantages and limitations of the activated sludge method follows:

Advantages:

1. The continuous and the semicontinuous methods using high concentrations of biomass simulate in the laboratory the treatment that wastewater normally receives.
2. There is considerable information on the characteristics of these units since the method has been used for many years.
3. Reproducibility is very good if the unit is operated until steady state is achieved.
4. Sample size is not limited.
5. The test compound does not have to be pure. If mineral salts medium is used, BOD or COD tests can be used to calculate an oxygen material balance.

Disadvantages:

1. Continuous operating units require money, space, and maintenance.
2. Single batch runs do not allow for acclimation unless the test period is unduly prolonged.
3. Residence time of 23 hours in the fill-and-draw method is not typical of most treatment plants, and therefore relates only to extended aeration.
4. Most investigators feel that valid results cannot be obtained in less than ten days. There are always unexplainable fluctuations in the efficiency of the process that must be averaged.
5. A chemical or physical analytical method must be available for the analysis of each compound if wastewater is used as feed.

Trickling Filter Method --

Many small towns use trickling filters as the means of secondary treatment. The name is a misnomer to the extent that the filter is not a filter in the ordinary chemical engineering connotation. The principle of the method is that the supernatant from the primary

settling tank is sprayed on a bed of crushed stones about 6 ft deep. Recently, plastic trickling filter medium has come into being. The wastewater trickles through the bed to under-drains that lead to a final settling tank. The action of the filter consists in mechanically removing the lighter solids not removed by sedimentation and in the oxidation of the wastewater by contact with the microbial film on the filter medium.

Experimental trickling filters have never been a popular device for measuring biodegradability. Small units require small size media that increase operational difficulties as a result of ponding, air circulation, and side wall effects. If recycling is not practiced, the most important dimension is the depth of the filter bed because that determines the retention time. The bed should be at least 6 feet deep. The main limitation is the time required to develop, acclimate, and equilibrate the biological film. It can be a matter of months before steady state is reached. If a trickling filter is used for biodegradability studies, the BOD or COD or TOC removals also should be determined so that a comparison can be made between the percentage removal of the other constituents of the wastewater and the test compound.

Section 2. Treatability of Specific Compounds

ANAEROBIC STUDIES

Of all the biological treatment processes, anaerobic digestion is one of the most susceptible to poisoning by toxic substances, especially heavy metals and chlorinated organic compounds. Anaerobic digestion is two biological processes rolled into one. The first group of bacteria degrade the starches, fats, and proteins to mostly short-chain fatty acids. The second group, methane-producing bacteria, continues the process of degradation to produce methane as the end-product. This group is much more susceptible to inhibition than the first. The reduction of methane production signals digester failure, which results in offensive sludge that is not well digested. The toxic effect of chlorinated organic compounds at very low concentrations is remarkable, and provides a warning that a close watch must be kept on the disposal of the new chlorinated hydrocarbons that come on the market. Toxic levels of a number of organic substances are shown in Table 1.

The effects of metals on anaerobic digesters are dependent on the amount of sulfides present. Sulfide precipitates heavy metals, thus forming insoluble metal sulfides that are not toxic. Most digesters contain enough sulfides that failures are not usually the result of metals except for large slug dosages from spills. Some of the more common metals and their acceptable concentrations in digesters are shown in Table 2.

Although the resilience of bacterial populations has often been stressed, this capability is more commonly observed in aerobic processes than in anaerobic digestion. Once the performance of an anaerobic digester has deteriorated, it is usually rather difficult to reestablish.

Anaerobic tests for biodegradation should be managed so that the test system has a hydrogen electrode (E_h) potential of about -0.4 volts, dissolved oxygen is excluded, and methane gas is generated. Because anaerobic organisms have protracted generation periods, time studies should extend to at least thirty days. If any change is made to the system, the observations of the effects should continue for at least three times the hydraulic detention time of the system.

TABLE 1. TOXICITY TO ANAEROBIC DIGESTERS

Organic compound	Concentration mg/l In wastewater	In sludge
Acrylonitrile	-	5
Carbon tetrachloride	-	10
Chloroform	0.1	-
1, 2—Dichloroethane	-	<1
Methylene chloride	1	-
Di(5—chloro—2—hydroxyphenyl)methane (dichlorophen)	1	-
Pentachlorophenol	0.4	-
1, 1, 1—Trichloroethane	-	1
Trichlorofluoromethane	0.7	-

TABLE 2. TOXICITY OF METAL IONS TO ANAEROBIC DIGESTION OF PRIMARY SLUDGE

Metal ion	Concentration in wastewater, mg/l
Cadmium	1
Copper	10
Dichromate (as Cr)	50
Lead	50—70
Nickel	40
Tin (as Sn)	9
Zinc	10

Sodium Salt of Nitrilotriacetic Acid

In 1971, the sodium salt of nitrilotriacetic acid was suggested as a replacement for the phosphates in detergent formulations. The material was found to be aerobically degradable, but reports of anaerobic degradation were conflicting. To resolve this issue, sodium nitrilotriacetic acid ($NTA \cdot Na_3$) was studied in the fill-and-draw apparatus shown in Figure 5. At various times in the study period, both primary sludge and waste-activated sludge served as feed to the anaerobic digester.

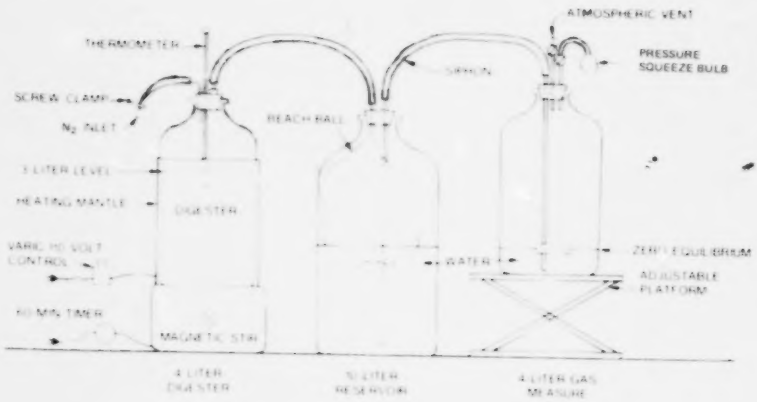


Figure 5. Digester apparatus.

The program was divided into three distinct study periods, the first of which was a control period to document the digester performance.

The second period concerned the degradability of NTA·Na_3 in an anaerobic system receiving only primary sludge feed. To initiate this study, 30 mg of NTA·Na_3 were added directly to the digester, yielding a concentration of 10 mg/l in the mixed contents. For an interval of one week, the primary sludge feed also contained 10 mg/l of NTA·Na_3. This was an acclimation period designed to indicate whether the 10 mg/l concentration would have any deleterious effect on the digestion process. The gas production and pH remained within normal limits. The NTA·Na_3 concentration in the daily feed sludge was then increased to 20 mg/l, and maintained at that level for three months. Figure 6 shows the NTA·Na_3 concentration in the mixed digester contents during this time period. On day zero the concentration of NTA·Na_3 was abruptly increased to slightly over 10 mg/l as a result of the direct addition of 30 mg of NTA·Na_3 to the digester. The NTA·Na_3 concentration gradually increased to the feed concentration 20 mg/l over a 120-day period. This would be predicted on the basis of the hydraulic detention time of 30 days. Over the 120 days of observation, no appreciable degradation of the NTA·Na_3 was noted in the primary sludge digestion process. Table 3 shows that the digester was performing satisfactorily with regard to organic loading, gas production, and volatile solids destruction during this second period. The conclusion was that NTA·Na_3 was not degraded in an anaerobic process operated entirely on primary sludge feed.

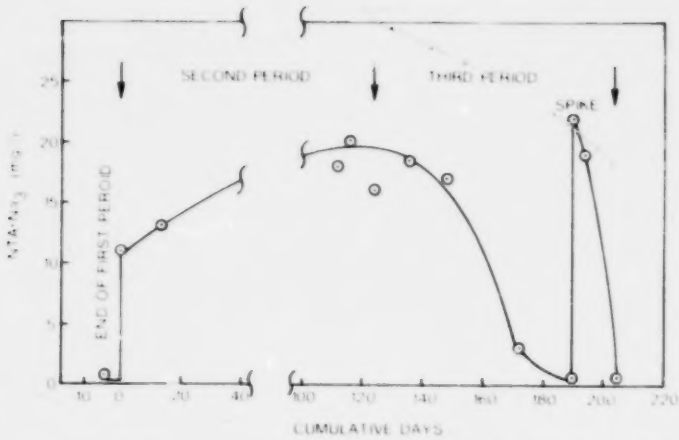


Figure 6. NTA·Na_3 concentration of digester, day 10 through 205.

TABLE 3. ANAEROBIC DIGESTER PERFORMANCE

Parameter	Study period		
	1	2	3
Volatile solids destroyed (%)	49	76	65
Volatile solids loading (g/l/day)	0.71	0.80	0.57
Gas produced from volatile destroyed (l/g)	0.89	0.94	0.95
Gas produced from volatile added (l/g)	0.43	0.72	0.62
Volatile solids in feed (%)	82	69	83

The third study period concerned the anaerobic digestion of primary and waste-activated sludge. The digester was switched to a daily feed of 80 percent primary sludge and 20 percent waste-activated sludge, on a volume basis. The overall concentration of NTA·Na_3 was 20 mg/l. The waste-activated sludge was obtained from an activated sludge plant that was acclimated to the degradation of NTA·Na_3. The digestion process was monitored for two weeks. Operation was satisfactory, and the concentration of NTA·Na_3 in the digester contents remained near 20 mg/l. The percent of waste-activated sludge in the daily feed was then increased to 50 percent by volume. Over a two-month period, the

$NTA \cdot Na_3$ content of the mixed digester contents gradually decreased, until at the end of this period the concentration was near zero (see Fig. 6). To verify that the anaerobic system was biologically degrading the $NTA \cdot Na_3$, a direct addition of $NTA \cdot Na_3$ (to yield about 20 mg/l in the mixed digester contents) was added on day 190. The usual daily dose of 20 mg/l in the combined sludge feed was continued. Figure 6 shows the response of the system as measured by the $NTA \cdot Na_3$ content of the reactor contents. In about 15 days (day 205), the residual $NTA \cdot Na_3$ was again near zero.

Table 3 provides a summary of the digester's performance during each of the three study periods. Typical anaerobic digester performance values were obtained in each period, a fact that indicates good operational control of the reactor.

The results of this study support the conclusion that primary sludge does not contain a microbial population that can multiply during anaerobic digestion and degrade $NTA \cdot Na_3$. However, once acclimated organisms are established in the mixed digester contents by means of an inoculum of facultative organisms from waste-activated sludge, anaerobic degradation of $NTA \cdot Na_3$ can proceed.

AEROBIC STUDIES

This section summarizes a variety of aerobic biodegradation test methods that have been used with specific classes of compounds.

Bacterial Utilization of Lignans

Lignans are a family of methoxylated aromatic compounds of known molecular composition that are structurally related to, but less complex than, lignins. Lignins are more complex methoxylated polymers that are extremely resistant to microbial action. Alpha-conidendrin is a representative of the lignan type molecule. Organisms capable of degrading this material were isolated from natural sources using adaptation techniques.

A synthetic basal salts medium containing 0.1 to 1.0 percent α-conidendrin as the sole carbon source was used for enrichment cultures.

Since α-conidendrin is insoluble, the analytical procedure used to detect degradation was determination of methoxyl groups on the residual solids at various times. Alpha-conidendrin has a methoxyl content of 17.5 percent. An adapted culture inoculated into an aerated culture medium containing 200 mg/l α-conidendrin reduced this value to 0.8 percent in 20 days. Figure 7 shows the daily progress of the degradation.

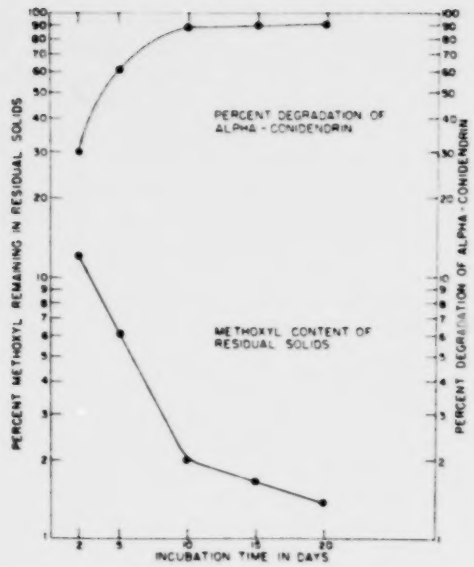

PERCENT METHOXYL REMAINING IN RESIDUAL SOLIDS
PERCENT DEGRADATION OF ALPHA - CONIDENDRIN
PERCENT DEGRADATION OF ALPHA - CONIDENDRIN
METHOXYL CONTENT OF RESIDUAL SOLIDS
INCUBATION TIME IN DAYS

Figure 7. Methoxyl content of residual solids and percentage degradation of α-conidendrin.

The organisms adapted to the utilization of α-conidendrin dissimilated vanillic, *p*-hydroxybenzoic, and protocatechuic acids when grown on chemically defined media with each of these acids as a sole carbon source. Cultures freshly isolated from a natural source by streaking on α-conidendrin agar when incubated in a medium with either vanillic, *p*-hydroxybenzoic, or protocatechuic acid as the only carbon source showed turbidimetric measurements indicating a limited amount of growth. However, when the same culture was subjected to many successive transfers in a liquid α-conidendrin medium, and was then inoculated into a medium with any one of the above acids as the sole carbon source, the amount of growth increased from 40- to 60-fold over that obtained in the same acids before the culture was adapted.

Adaptation studies, together with chromatographic and spectrophotometric analyses of the degradation products, indicate that the metabolic pathway in the dissimulation of α-conidendrin by pseudomonad strains may proceed from α-conidendrin ---→ vanillic acid ---→ *p*-hydroxybenzoic acid ---→ protocatechuic acid ---→ keto-adipic acid.

Degradation of Aromatic Compounds by Phenol-Adapted Cultures

A culture acclimated to 300 mg/l phenol was tested in Warburg respirometers to determine its potential for degrading 104 aromatic compounds. The washed culture was added to 0.067 M phosphate buffer contained in the respirometers and the test compound was introduced from the side arm to yield a 100 mg/l concentration. Oxygen uptake was measured and chemical colorimetric analyses were done for the specific aromatic compounds. Observations were carried out for about 200 minutes, with the oxygen measurements made at 10 minute intervals.

The various aromatic groups tested were phenols, benzyl alcohols, hetrocyclics, cyclics, benzoic and other acids, benzaldehydes and benzamides, and substituted benzenes. The results are shown in Figures 8, 9, 10, 11, 12, and 13.

On the basis of the results for these organic compounds, there appeared to be a relationship between molecular structure and resistance to bacterial degradation. The results indicated significant differences in resistance to oxidation within each of several well defined groups of compounds. The relationship between molecular structure and ease of degradation was also apparently affected by the position of a group on the ring, the type of group, multiples of the same or different substituents, and the size and complexity of the substituent. The results indicated that there was a possible relationship between molecular structure and ease of degradation of some of the phenols. The presence of more than two hydroxyl groups on the ring appeared to make a compound highly resistant to oxidation, for example, phloroglucinol and pyrogallol. Dihydric phenols seemed to be oxidized to about the same, or possibly a somewhat lesser degree than phenol. The fact that none of the nitrophenols tested, except *o*- and *m*-nitrophenol, and 2,4-dinitrophenol, showed any appreciable O_2 uptake indicated that adding a nitro group tended to increase resistance.

Chloro substitution showed that dichlorophenols were more resistant to oxidation than monochlorophenols. The effect of the methyl group was indicated by the relatively high level of activity with cresols and with some dimethylphenols. The effect of position of the substitution on the ring was illustrated with the cresols and dimethylphenols. With cresols, substitution of the methyl group in the para position resulted in a higher initial O_2 uptake rate, but a lower total O_2 uptake, than was the case with *o*- and *m*-cresol. The same effect for para-substituted compounds was observed with dimethylphenols. High total O_2 uptakes were noted with 2,4- and 3,4-dimethylphenol, whereas oxidative activity was lower with dimethylphenols having methyl groups in other positions. All monochloromethylphenols had a measurable O_2 uptake while

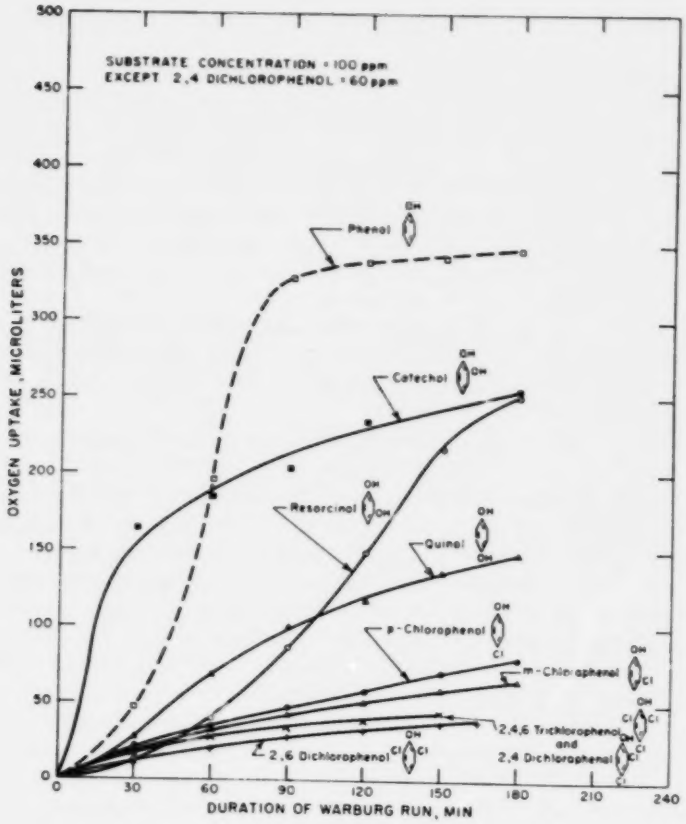


Figure 8. Oxidation of hydroxy- and chlorophenols.

there was little activity with a dichloromethylphenol (4,6-dichloro-*m*-cresol). This indicated that, as with the chlorophenols, dichloro substitution increased the resistance of chloromethylphenols. The presence of a methoxyl or phenoxyl group on the ring also increased resistance to degradation.

Differences in O_2 uptake related to molecular structure and substitution were also observed with benzoic acids. Activity with benzoic acid was relatively higher than with its hydroxyl derivatives. The latter in turn had greater activity than the trihydroxyl derivatives. In general, the hydroxybenzoic acids were more susceptible to degradation than the other substituted benzoic acids. The influence of position of substitution was evident in the case of methylbenzoic acids because *m*-toluic acid was more readily oxidized than the ortho and para derivatives. With methoxylated

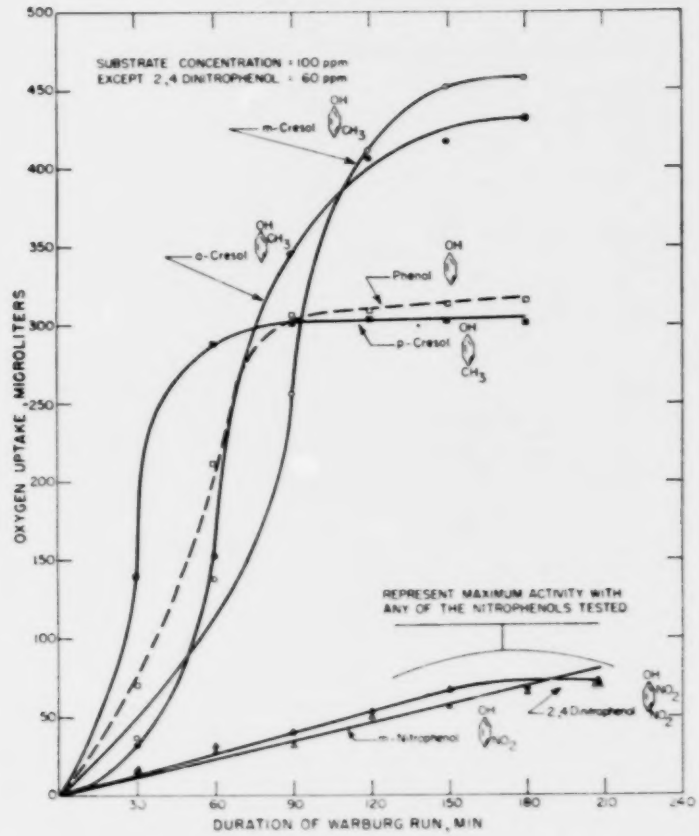


Figure 9. Oxidation of methyl- and nitrophenols.

benzoic acids, increasing the number of methoxyl groups on the ring apparently interfered with oxidation. Syringic acid was more resistant to degradation than vanillic acid. The presence of two carboxyl groups significantly reduced the rate of O_2 uptake by the benzoic acids. In the case of hydroxybenzoic acids and nitrobenzoic acids, the number of compounds tested appeared to be sufficient to provide some statistical basis for tentatively concluding that the former were readily oxidized by the phenol-adapted bacteria, whereas the latter were extremely resistant.

A high level of oxidation was observed with benzaldehyde and its para hydroxyl derivative. Replacement of the hydroxyl by a nitro group blocked the activity. This was demonstrated by the

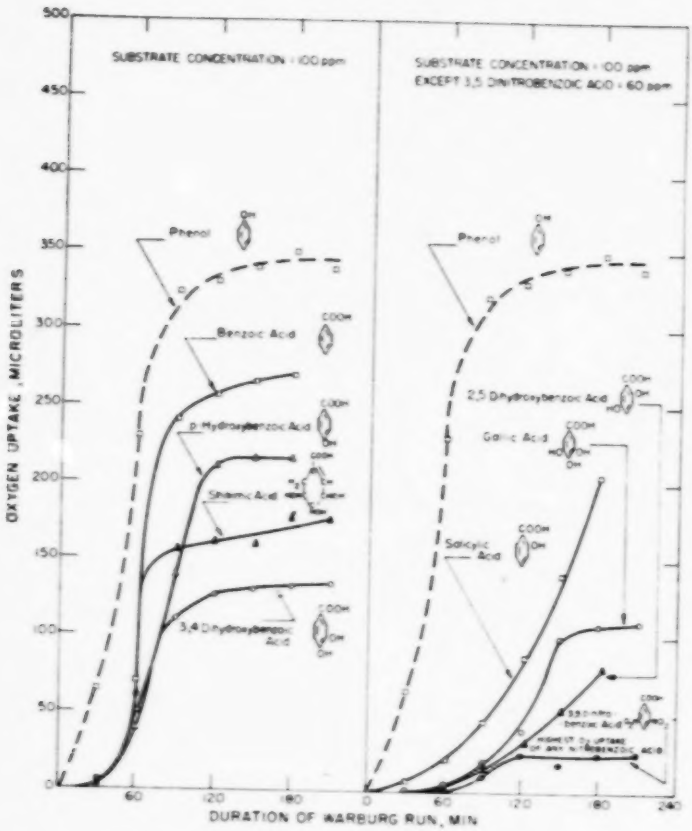


Figure 10. Oxidation of benzoic acids.

resistance of *m*- and *p*-nitrobenzaldehydes. Benzamide, a compound in which an NH_2 group replaces the hydrogen in the CHO group of benzaldehyde, is also resistant. A measurable level of activity was observed with vanillin.

Results obtained with benzene and its chloro derivatives, regardless of the number of position of the chloro substitutions, showed that, in general, these compounds were resistant to degradation. The effect of a nitro group was significant, as indicated by such compounds as nitrobenzene, and *m*- and *p*-dinitrobenzene, which had a low level of activity. In particular, the activity of the mononitro-substituted benzene was below the endogenous level. The

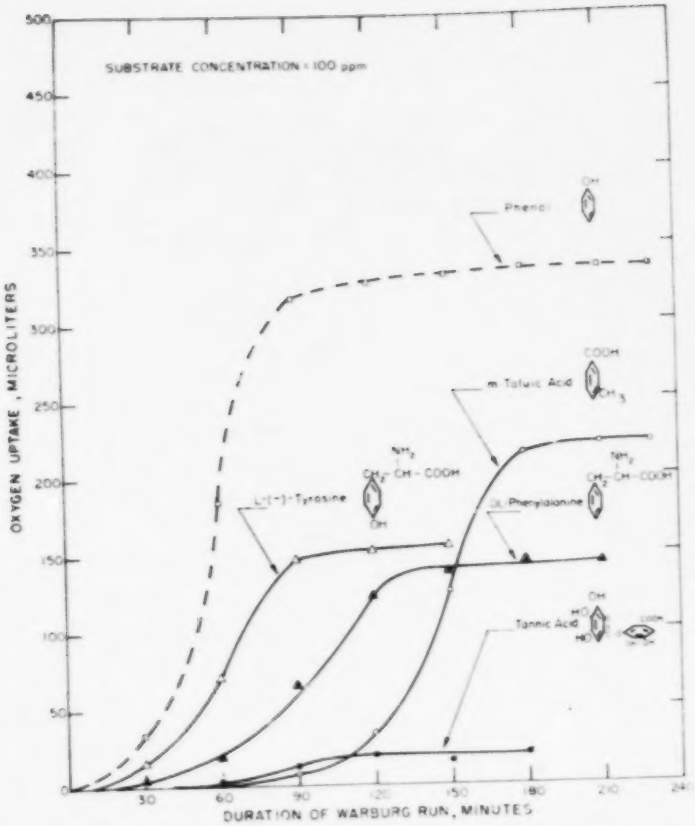


Figure 11. Oxidation of selected benzoic and amino acids compared with that of tannic acid.

resistance of the nitrobenzenes to degradation seemed to decrease as the number of nitro groups on the benzene ring increased. Neither the methyl nor amino group appeared to interfere completely with the oxidation of benzene because both aniline and toluene were oxidized to a limited extent. The position of the nitro substitution may be important because there was evidence of some oxidation of *m*-nitroaniline but little, if any, activity with *p*-nitroaniline. Both 2,4-dinitrotoluene and 2,4,6-trinitrotoluene (T.N.T.) appeared to be oxidized to some extent.

Studies of this type are useful for the preliminary screening of a large number of compounds. The occurrence of oxidation under these conditions indicates that the compound would be oxidized

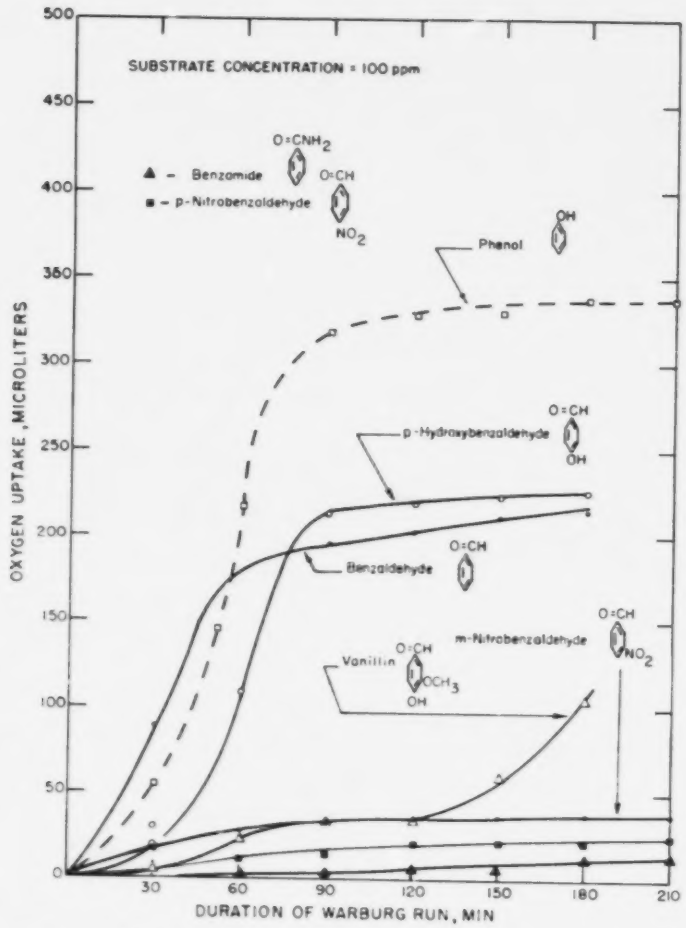


Figure 12. Oxidation of benzaldehydes and benzamides.

in municipal biological treatment plants. Failure of a compound to be oxidized under these conditions is not conclusive since in actual practice microbial population selection could occur.

Organic Compounds for Replacement of Phosphates in Detergent

Formulations --

The major function of phosphate in detergent formulations is to prevent precipitation of calcium salts during laundering. As a

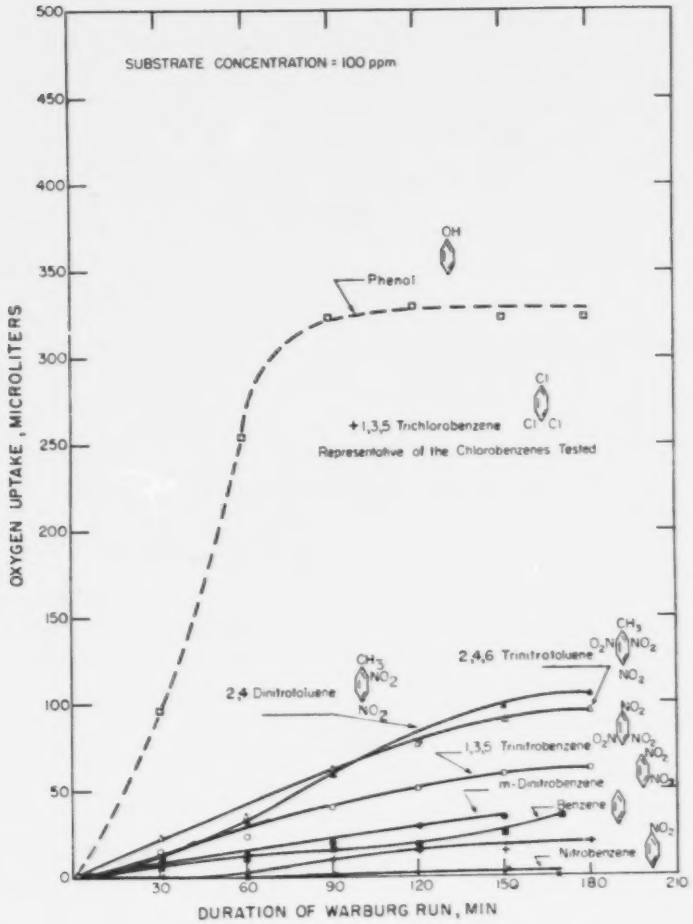
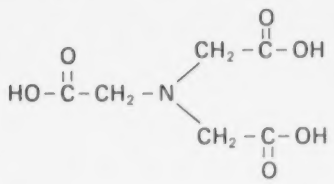


Figure 13. Oxidation of benzenes.

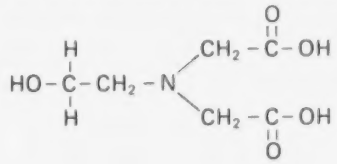
result of the relationship between excessive phosphate discharges and eutrophication, several organic compounds have been suggested as substitutes for phosphates.

In 1967, the compounds shown in Figure 14 were candidates for consideration as phosphate substitutes. These six materials were tested for biodegradation using a basal salts medium with yeast extract and an inoculum of settled wastewater. The compounds were tested at concentrations of 5, 10, and 20 mg/l. The flasks

NITRILOTRIACETIC ACID (NTA)



HYDROXYETHYLIMINODIACETIC ACID (HEIDA)



DIETHYLENETRIAMINEPENTAACETIC ACID (DTPA)

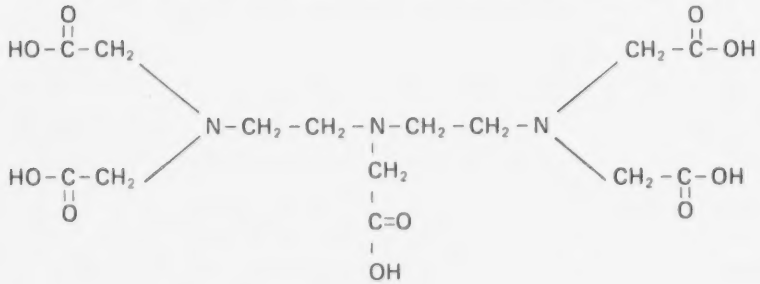
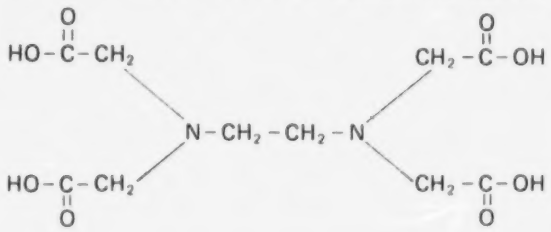
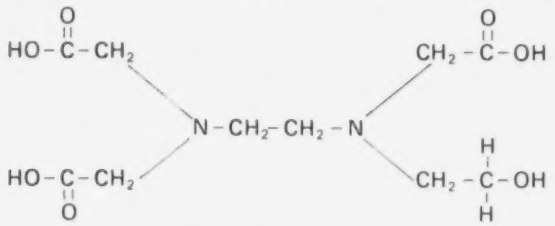


Figure 14. Structural formulae of complexones.

ETHYLENEDIAMINETETRAACETIC ACID (EDTA)



N-HYDROXYETHYLETHYLENEDIAMINETRIACETIC ACID (HEDTA)



1, 2-DIAMINOCYCLOHEXANE-N, N'-TETRAACETIC ACID (DCTA)

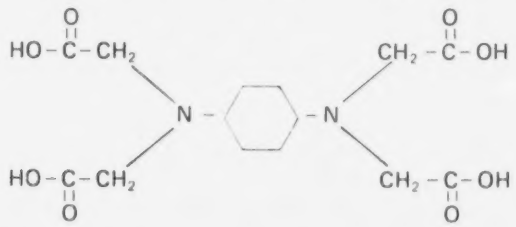


Figure 14. (cont'd). Structural formulae of complexones.

remained static and weekly serial transfers were made for three consecutive weeks. After incubation for seven days, each flask was analyzed for the test compound to detect the amount of degradation. The chemical method used to determine the undegraded chelating agent was a complexation titration at pH 2.5 with copper sulfate using PAN (1-(2-pyridylazo)-2-naphthol) as the indicator. Each compound listed was run on five different occasions to eliminate any variability of the inoculum. The biodegradable compounds showed biodegradability in every test.

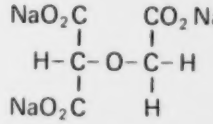
This method for testing for biodegradability provides an indication of the time required for adaptation, as well as a determination of whether a compound is degraded during the test period.

Only NTA (nitrilotriacetic acid) and HEIDA (hydroxyethyliminodiacetic acid) lost their chelating ability under the test conditions. The acclimation period for NTA was about two weeks; that for HEIDA was slightly less. Both these compounds contain only one nitrogen atom; the other four compounds, containing two nitrogen atoms, did not degrade under these test conditions.

Although the analytical method only determined whether the compound lost its chelating ability, it would be fairly safe to assume that fragments of these molecules would be subject to complete degradation. No manometric testing was done.

Another organic compound under consideration as a phosphate replacement is carboxymethyl tartronate, more commonly referred to as CMT. This compound has the formula:

(Trisodium 20-oxa-1,1,3-propane tricarboxylate)



The biodegradation of this compound at a concentration of 20 mg/l was studied in 300 ml continuous flow reactors as shown in Figure 9, using a mineral salts medium with yeast extract and supplemental organic materials. The results are shown in Figure 15. The remarkable feature is that a period of about 12 weeks was necessary for acclimation. Attempts to shorten the acclimation period by external addition of forest and garden soil were not successful. However, once acclimated, the culture could degrade shock CMT doses, and could immediately degrade CMT after a substrate starvation period.

During this study three analyses were used to monitor the CMT in the reactor effluent: TOC, a non-specific colorimetric, and a specific gas chromatographic procedure. After acclimation, the test reactor and the control reactor effluent had about the same TOC concentration. Figure 16 shows the comparison of the colorimetric and the gas chromatographic assays during 18 weeks of the test period.

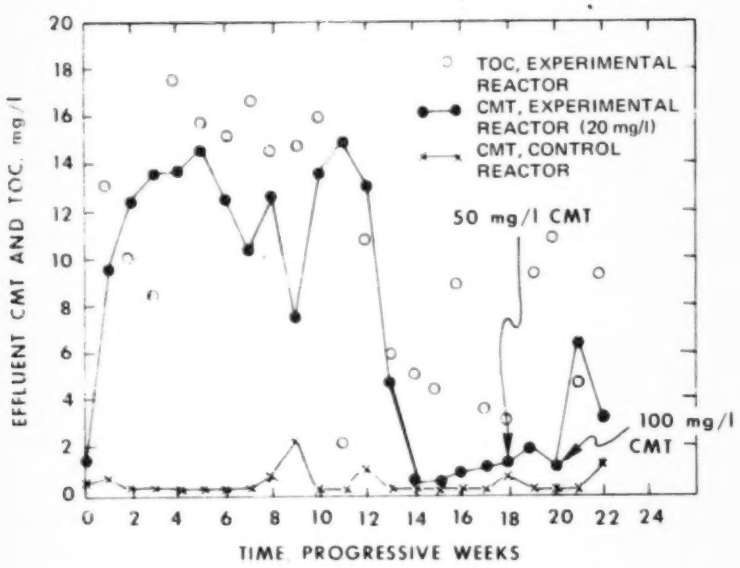


Figure 15. CMT and TOC of reactor effluents with synthetic feed.

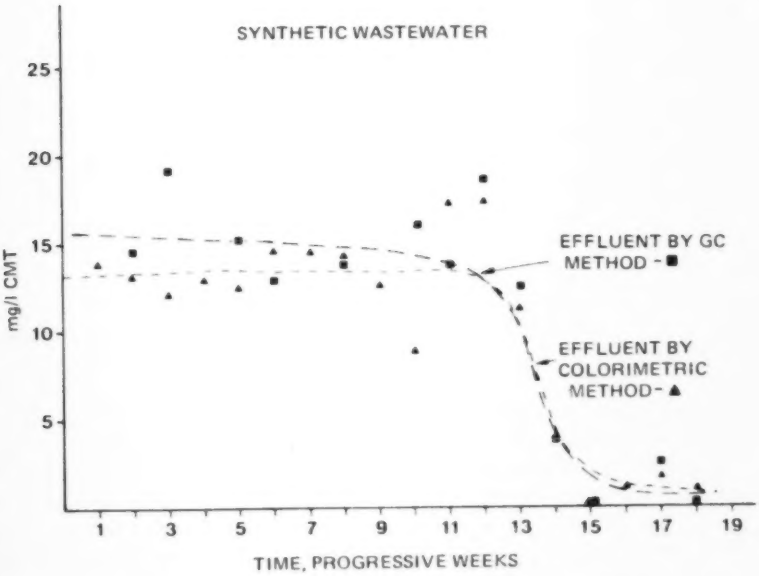


Figure 16. Comparative analytical methods for CMT in reactor effluent.

Synthetic Detergents --

In the early 1960s great attention was focused on the biodegradability of synthetic detergents because of the occurrence of billows of foam at wastewater treatment plants and along stretches of rivers. Early production of detergents yielded compounds with branching hydrocarbon chains that were relatively resistant to biodegradation. Various alternative compounds were considered for replacement.

Using a static mineral salts medium with added yeast extract, many of these compounds were screened for biodegradability. Provision for acclimation was a part of the protocol. Settled wastewater was used to seed the medium. Tests on each compound were repeated on five different occasions to determine the variability of the seed. Table 4 gives the average results of these five replicate tests for various synthetic detergents. Several phenolic compounds with known degrees of degradability were included to verify the method. The compounds were tested at a concentration of 10 mg/l.

The chemical analysis used to detect the undegraded alkyl benzene sulfonates and alkyl sulfates was the methylene-blue method. The cobalt-thiocyanate test procedure was used for the nonionic surfactants. Details of this method can be found in Reference 10. Indirect determinations also were made by judging the foaming tendencies of the surfactants. The loss of surface activity or foam does not necessarily mean complete degradation of the molecule. The 4-amino-antipyrene method was used for the assay of phenol, *o*-cresol, and the chlorinated phenols. The nitro compounds were adjusted to pH 10.0 and determined spectrophotometrically. As a further check on the phenolic compounds, acid was added to all the terminal flasks, ether extracted, and the ether examined for the benzene ring in the ultraviolet region of the spectrum. No suitable solvent was found that would extract polyethylene glycol 400 from the medium; therefore, it was necessary first to dry the contents of the flasks at 80° C before using the cobalt-thiocyanate procedure. The ether extraction step was omitted and the complex formed directly with the dried residue.

The results obtained demonstrate the general applicability of this test. In addition to determining whether a compound is degraded during the test period, it provides an indication of the time required for adaptation. This is very useful information because it is not enough merely to know that a compound is degradable; the rate at which it is degraded is equally important where water quality depends on it. Such information is helpful in predicting the behavior of a compound in a waste treatment plant and in surface waters. For example, if microorganisms in a stream or waste

TABLE 4. DEGRADABILITY OF SURFACTANTS

Name of compound	Percent of compound degraded			
	Number of subculture			
	Initial	1	2	3
Anionic surfactants:				
Linear alkyl (C_{12}) benzene sulfonate	84.5	98.5	99.0	99.0
Linear alkyl (C_{10}—C_{15}) benzene sulfonate	90.5	90.5	92.5	92.0
Linear alkyl (C_{12}—C_{14}) benzene sulfonate	93.5	97.0	97.0	97.0
Primary (C_{12}) alcohol 3-mole ethoxysulfate	99.5	99.5	99.5	99.5
Secondary (C_{11}—C_{15}) alcohol 3-mole ethoxysulfate	96.0	97.0	97.0	97.5
Linear alkyl (C_{14}) benzene sulfonate	61.0	92.5	96.0	96.0
Linear alkyl (C_{11}—C_{15}) phenol 5-mole ethoxysulfate	39.5	12.5	11.5	12.0
Branched alkyl (C_9) phenol 4-mole ethoxysulfate	9.5	9.0	3.5	7.0
Nonionic surfactants:				
Linear primary (C_{14}) alcohol 8-mole ethoxylate	98.5	99.0	99.0	99.0
Linear primary (C_{12}) alcohol 9-mole ethoxylate	99.5	99.5	99.5	99.5
Polyethylene glycol - 400	52.0	42.0	63.5	61.0
Branched alkyl (C_8) phenol 9-mole ethoxylate	0.0	0.0	0.0	0.0
Branched alkyl (C_9) phenol 15-mole ethoxylate	12.5	6.5	0.0	0.0
Branched (C_{13}) alcohol 9-mole ethoxylate	1.5	0.0	2.0	2.0
Phenol	99.5	99.5	99.5	99.5
o—Cresol	99.5	99.5	99.5	99.5
o—Nitrophenol	81.0	84.5	93.5	98.5
2,4—Dichlorophenol	8.0	47.0	91.5	98.5
m—Chlorophenol	0.0	0.0	0.0	0.0
2,4,6,—Trinitrophenol	0.0	0.0	0.0	0.0

treatment plant were exposed continuously to a compound such as 2,4-dichlorophenol, the microorganisms probably would adapt to degrade it. If, on the other hand, the exposure is intermittent, such as a spill, one would not expect the compound to be degraded in a waste treatment plant. The compound might persist in a stream for days before the naturally occurring microorganisms adapted to degrade it.

As a result of the public concern about nondegradable detergents, leading manufacturers modified their production processes to develop detergents with linear hydrocarbon chains. The marketing of these new materials began about 1965. Five treatment plants located around the nation were monitored to determine the change in influent wastewater surfactant quality. Monthly raw wastewater samples were collected for 18 months. One liter samples were aerated in 2-liter flasks for seven days after inoculation with 40 mg of volatile solids from an activated sludge aeration tank. The determination of the ratio of hard detergent (resistant to degradation) to soft detergent (readily degraded) was calibrated by experimental procedures using various mixtures of known lots of hard and soft detergents. Methylene-blue active substance (MBAS) was the analytical method used for determining degradation.

The data accumulated for one of the plants, in Richmond, Indiana, are given in Figure 17. Similar results were noted at the other four sites. It was concluded that a vigorous manufacturing and marketing strategy had been deployed and the quality of surfactants in influent wastewater rapidly changed from the undesirable hard form to the acceptable soft form.

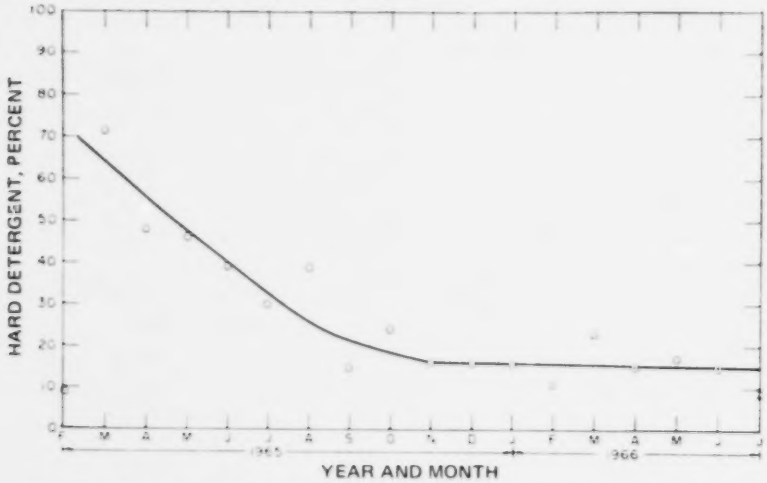


Figure 17. Percent hard detergent in a raw wastewater; Richmond, Indiana.

Steroid Hormones

The increased medical use of natural steroid hormones, coupled with their recent use as oral contraceptives has raised concern about their persistence in wastewater and the ability of conventional waste treatment processes to remove these compounds. The normal concentration of steroids in municipal wastewater in the United States would not be expected to be more than a few tenths of a milligram per liter. Nevertheless, since they are physiologically active in very small amounts, it is important to determine to what extent the steroids are biodegraded in the normal course of wastewater treatment and in the receiving bodies of water that may eventually be used for water supplies.

There is little information on the biodegradation of steroid hormones. The literature is almost void of studies that report ring cleavage and complete oxidation of the molecules. A few studies have been done on some of the natural steroids, but no biodegradation studies of the synthetic ovulation-inhibiting hormones have been reported.

Hormones representing different groups of steroids were selected for the biodegradability studies. The following is a list of representative hormone compounds from each of the classes of steroids chosen for the study: (1) Sterols – cholesterol; (2) Adrenocortical hormones – progesterone and pregnanediol; (3) Estrogenic hormones – estrone, beta-estradiol, and estriol; (4) Androgenic hormones – testosterone and androsterone; (5) Androgenic hormone derivative – methyl testosterone; and (6) 17-ketosteroids – estrone and androsterone. The most commonly used synthetic sex hormones were used for these studies. They were: (1) Estrogens – ethynyl estradiol and mestranol (ethynyl estradiol-3-methyl ether) and (2) Progestins – norethynodrel (17-hydroxy-19-nor-17α-pregn-5(10)-en-20-yn-3-one), norethindrone (17-hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one), dimethisterone (6α,21-dimethyl 17-hydroxy-pregn-4-en-20-yn-3-one), etyhnodiol diacetate (19-nor-17α-pregn-4-en-20-yne-3β,17-diol), norethindrone acetate, medroxyprogesterone acetate (17-hydroxy-6α-methyl-pregn-4-ene-3,20-dione acetate) and chlormadinone acetate (6-chloro-17-hydroxy-pregna-4,6-diene-3,20-dione).

The compounds were tested at a concentration of 20 mg/l in a basal salts medium plus yeast extract, using activated sludge organisms as inoculum. The flasks were aerated by shaking during the entire observation period. Since these compounds are insoluble, they were suspended in the medium by introducing the hormone dissolved in acetone and warming the medium to evaporate the acetone.

Generic colorimetric assays for each group of steroids were used to follow the progress of the degradation. The fate of the compounds was also determined by spectrophotometric scanning to determine ring cleavage, and use of ascending thin layer chromatography for identification of residual products. Because the materials are insoluble, both the supernatant and solids portion of the medium had to be extracted with chloroform to account for the total steroid content of the flask. Each compound was evaluated three times in duplicate. Provision for acclimation was made by transferring subcultures into fresh medium weekly. Table 5 shows the average percent degradation for the three test runs on each steroid.

TABLE 5. AVERAGE PERCENT LOSS OF STEROIDS BY WEEKLY SUBCULTURE ENRICHMENT

Steroid compound	Percent degradation of compound				
	Number of subcultures				
	Initial	1	2	3	4
Androsterone	96	100			
Cholesterol	96	100			
Testosterone	95	100			
Progesterone	93	100			
Estrone	91	98	100		
Beta-estradiol	90	97	100		
Estriol	82	96	100		
Pregnanediol	79	95	100		
Methyl testosterone	77	91	97	100	
Ethynyl estradiol	72	89	97	100	
Norethynodrel	71	88	97	100	
Norethindrone	70	87	96	100	
Norethindrone acetate	68	84	95	100	
Dimethisterone	65	82	93	99	100
Medroxyprogesterone acetate	56	80	92	98	100
Chlormadinone acetate	55	78	91	97	100
Ethynodiol diacetate	52	72	90	94	96
Mestranol	47	73	88	89	90

The overall results of these studies show a variation in the relative susceptibility of the natural hormones and the synthetic estrogen and progestin components of oral contraceptives to biodegradation. They indicate that in the microbial population found in activated sludge, there are species capable of producing enzymes that will degrade both natural and synthetic steroid hormones if conditions for acclimation are provided.

Hazardous Organic Compounds

Benzidine (1,1'-biphenyl)-4,4'-diamine) a bicyclic arylamine, has been demonstrated to be carcinogenic in experimental studies with animals such as rats, mice, dogs, and hamsters. Literature citations implicate many arylamines including benzidine as causative agents of bladder carcinomas in humans. Because of this carcinogenic property, benzidine has been included in the list of "14 Carcinogens" tabulated in the Federal Register. The Occupational Safety and Health Agency has established standards to control worker exposure to these compounds, which are used in various process industries. Benzidine is used in hospitals, research laboratories, and dyestuff industries.

Even though the use of materials such as benzidine is regulated, it is advisable to ascertain the biodegradation of this compound in conventional wastewater treatment, and to determine what protection a secondary facility could afford a receiving water in the event that this material is discharged into a sewer system. Studies have shown that the majority of carcinogenic compounds, because of their strain-free condensed nuclear configuration, are resistant to bio-oxidation by unacclimated microorganisms in activated sludge.

The purpose of this study was to find whether or not acclimation to benzidine could be established in an activated sludge process operated in a continuous feed mode within a specific range of feed concentrations of benzidine under appropriate operating conditions.

Continuous flow reactors of the type shown in Figure 4 were used. The medium was settled municipal wastewater spiked with selected concentrations of benzidine. The reactors were operated at a hydraulic retention time of 24 hours and a solids retention time of 30 days. Phase I concerned benzidine concentrations of 0.5, 1, and 5 mg/l. Phase II studied benzidine concentrations of 10, 20, and 30 mg/l. Biodegradation was followed by a specific colorimetric assay of benzidine, utilizing oxidation with Chloroamine-T, and residual chemical oxygen demand analysis.

Since the biological solids were in the range of 4,000 mg/l, the sorption of benzidine to these active surfaces had to be evaluated. The solids for the 0.5 mg/l benzidine reactor contained 0.003

percent benzidine by weight. Solids from the 30 mg/l reactor contained 0.3 percent sorbed benzidine. On a mass through-put basis, both these determinations show that only about one percent of the dosed material was sorbed to the biological floc.

The color of the reactor sludges during Phase II was remarkable. The control unit mixed liquor was light tan in color during the entire study. At the 6-week point the 10 mg/l, 20 mg/l, and 30 mg/l dosed sludges were brown, dark brown, and brown-black, respectively. The intermediate oxidation products and benzidine itself are highly chromophoric compounds; and since these higher concentrations were not completely degraded, this visual observation confirms the analytical results.

Figure 18 presents the observations collected during the 2 phases of study.

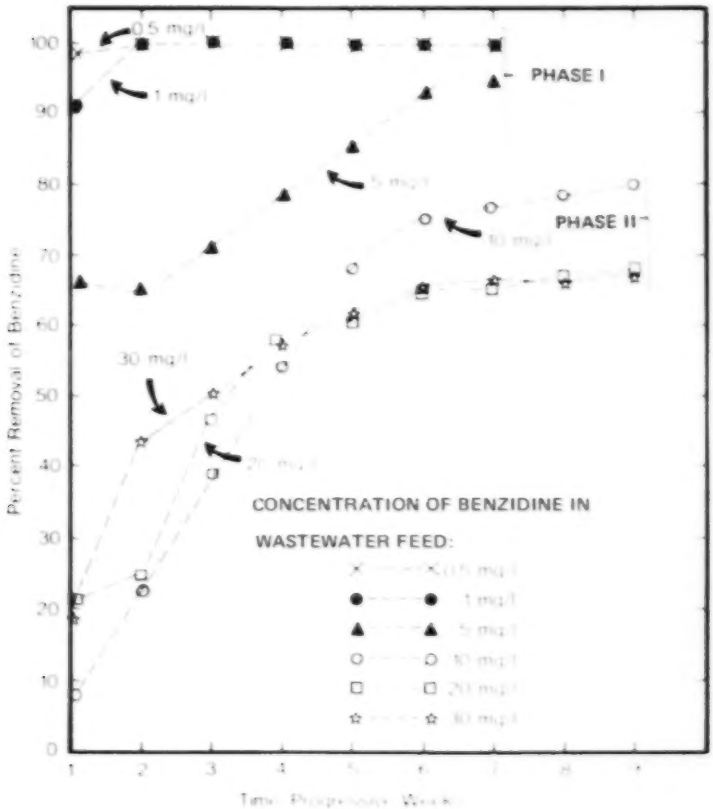


Figure 18. Acclimation to benzidine.

Results from the biodegradation studies on the several concentrations of benzidine show that acclimated extended aeration sludges can degrade continuous doses of 1 mg/l benzidine. Less complete degradation occurs at higher dosage levels and intermediate oxidation products begin to accumulate in the system. Even at the highest dose of benzidine, 30 mg/l, no marked interference with carbonaceous removal efficiency was noted, since the increased COD of this effluent could be related to the presence of undegraded benzidine.

Concentrations of benzidine between 1 and 5 mg/l were not studied, but because these studies employed an extended aeration process and most secondary municipal facilities have far shorter hydraulic retention times, it is recommended that influent benzidine concentrations not exceed 1 mg/l. This limit would ensure that unacclimated systems would not discharge significant concentrations of benzidine into receiving waters.

The work also suggests that ambient concentrations of benzidine below 1 mg/l will be degraded by natural ecosystems, and the material will not accumulate.

Two other compounds of environmental concern are acenaphthene (ANT) and dimethyl phthalate (DMP). Phthlate esters have been recovered from many environmental samples. These compounds are used as plastisizers and industrial intermediates. Some studies have indicated that these compounds bioaccumulate in plants and animals and subtle toxic effects have been noted. Naphthalene compounds have also been noted in a wide variety of environmental samples. Recent studies have shown naphthalene compounds to be toxic to aquatic invertebrates and fish. Knowledge of the biodegradation of these two compounds would be helpful in determining the efficiency of municipal treatment processes for control of the substances.

The compounds were studied in the continuous flow reactors shown in Figure 3. Settled wastewater was used as feed and ANT and DMP were added singly and in combination. Concentration levels of 0.1 and 1.0 mg/l were selected. It was possible to study ANT and DMP in combination because the compounds could be resolved using a Perkin-Elmer # 900 gas chromatograph equipped with a dual flame ionization detector and a Varian Series Number 4 computer data programmer.

The reactors were started with activated sludge and feed settled wastewater for one week prior to the experimental run. Seven reactors were operated at a hydraulic retention time of 6 hours and a mixed liquor suspended solids concentration of 3,000 mg/l. The ANT and DMP content of feeds and effluents was determined by extraction of the samples with Freon-TF and evaporation of the extract by means of the Kuderna-Danish evaporation procedure. The gas chromatograph could detect 5 μg of ANT or

DMP. Table 6 presents the results.

Reactors 1 and 2 were fed approximately 0.1 and 1.0 mg/l ANT, respectively; Reactors 3 and 4, 0.1 and 1.0 mg/l DMP; Reactors 5 and 6, 0.1 and 1.0 of both ANT and DMP; Reactor 7 was a control. ANT is insoluble in water and had to be dissolved in ethyl alcohol. The proper amount of 2 percent alcoholic stock solution was added to the wastewater feed to achieve the desired concentration.

Table 6 shows that after 48 hours of operation no ANT or DMP could be detected in the final effluents of any of the seven reactors. Trace amounts were noted in the first 24 hour sample. The TOC contents of the feeds to Reactors 2 and 6 are elevated above the other reactors because of the addition of the alcoholic solution to achieve 1 mg/l ANT.

The test procedure was replicated once each week for five weeks. In no case was either ANT or DMP noted in the effluents collected after 48 hours of operation.

TABLE 6. BIODEGRADATION OF ANT AND DMP

Reactor number	1	2	3	4	5	6	Control
Wastewater feed, mg/l:							
ANT	0.13	0.97	-	-	0.10	0.93	-
DMP	-	-	0.08	0.89	0.10	0.85	-
TOC	19	35	18	17	20	38	17
TKN	13	13	12	14	12	11	11
NO_3—N	0.3	0.1	0.1	0.1	0.1	0.1	0.1
Reactor effluent, 24 hr, mg/l:							
ANT	0.00	0.02	-	-	0.00	0.01	0.00
DMP	-	-	0.00	0.01	0.00	0.01	0.00
TOC	3	7	3	5	6	5	3
TKN	1	1	1	2	1	0.09	0.8
NO_3—N	10	9	11	13	10	9	8
Reactor effluent, 48 hr, mg/l:							
ANT	0.00	0.00	-	-	0.00	0.00	0.00
DMP	-	-	0.00	0.00	0.00	0.00	0.00
TOC	5	7	3	3	4	7	4
TKN	0.8	1	1	0.8	0.9	0.8	0.7
NO_3—N	10	8	10	13	10	8	9

To ensure that the aeration solids did not sorb the ANT or DMP, after one test period the reactor contents were extracted with Freon-TF. Insignificant sorption was noted. It is also unlikely that the compounds were air-stripped from the reactors, on the basis of the physical properties of ANT and DMP.

Table 6 shows that ANT and DMP singly or in combination at concentrations of 0.1 or 1.0 mg/l do not interfere with the oxidation efficiency of the microorganisms. The effluents contained low residual TOC and nitrification was not inhibited.

Section 3. Treatability of Metals by Activated Sludge Systems

Extensive treatability studies on the interaction of metals with activated sludge systems have been conducted at the U.S. Public Health Service's Robert A. Taft Engineering Center in Cincinnati.

Continuous flow pilot plants with a flow capacity of 750 liters per day were utilized. The design features of the pilot plants are shown in Figure 19. This size pilot unit is sufficient to allow study of chemical distribution, biological response, and engineering design features.

These pilot units proved to be good simulants of wastewater treatment processes. They were operated and maintained on a 24 hour, 7 day per week schedule, with sustained analytical supervision. Sufficient observations were made to establish statistically valid evidence of performance in systems with metal input and metal withdrawal in general working balance. Over a 5 year period, chromium, copper, nickel, and zinc were studied individually and in combination.

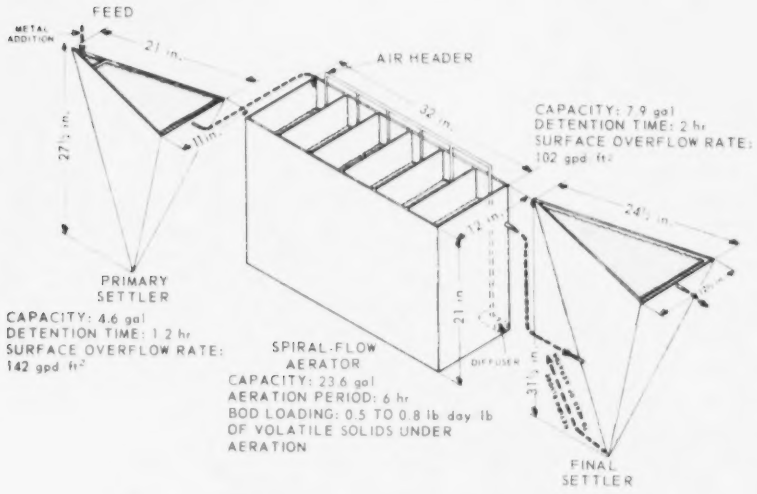


Figure 19. Plastic activated sludge pilot plant.

The wastewater feed to the plant during the various studies was either a weak supplemented domestic wastewater or a strong nonsupplemented domestic wastewater. Both feeds give results indistinguishable by the usual analytic measures.

DISTRIBUTION OF METALS THROUGH THE PROCESS

Complete material balances of the metals were made during each study. Table 7 summarizes these studies. The table is based on the amount of metal fed to a unit during a compositing period. Variation between compositing periods was common, as indicated by the range of observations for the efficiency of the process in removing metals. The percent metal unaccounted for in Table 7 is not a firm figure, but represents the cumulative errors involved in sampling sludges, flow measurements, and analytical methods.

Metal balances were performed for each selected concentration of the metals studied, not just those shown in Table 7. Each metal was studied in about five increments over the range of 1 to 20 mg/l. In addition, four metals were simultaneously traced during a combination study. Over the concentration ranges studied, no great difference in the efficiency of the process in removing the metals was noted. Zinc and copper, studied as the cyanide complexes, showed the same overall removal as when studied as the sulfates.

Chromium, introduced to an activated sludge process as hexavalent chromate, can show wide variation in concentrations at the various process outlets. Reducing substances in the raw sewage can cause precipitation of trivalent chromium with the primary sludge. Also, under anaerobic conditions, the organisms in the return sludge entering the primary settler can utilize the oxygen of the chromate radical and adsorb the trivalent chromium on the biological floc. Under these conditions chromium removal can reach 90 percent.

TABLE 7. DISTRIBUTION OF METALS THROUGH ACTIVATED SLUDGE PROCESS WITH CONTINUOUS DOSAGE

	% of metal fed			
Outlet	Chromium (VI) (15 mg/l)	Copper (10 mg/l)	Nickel (10 mg/l)	Zinc (10 mg/l)
Primary sludge	2.4	9	2.5	14
Excess activated sludge	27	55	15	63
Final effluent	56	25	72	11
Metal unaccounted for	15	15	11	12
Average efficiency of process in removing metal	44	75	28	89
Range of observations	18–58	50–80	12–76	74–97

Table 7 shows that a considerable portion of the metal introduced is removed in the secondary sludge. The effects of the metals on the mixed liquor are apparent even in the 1 to 2 mg/l range. During 5 years of study no bulking was encountered in a metal-fed system. The floc in the final settler quickly settled. Control units frequently bulked. Table 8 shows the effects of a combination of four metals on the sludge density index and volatile solids content of mixed liquor.

With the exception of zinc, the conventional activated sludge process is not very efficient in the removal of metals from the influent stream. The metal removed is concentrated at two points. In the primary sludge, a maximum concentration would occur if all the metal were removed with this sludge. Here the ratio of total flow volume to primary sludge volume is a limiting factor. Another point of concentration is in the secondary sludge. Since the volume of secondary sludge removed from the process may be small compared to the flow through the process, concentration may be high at this point.

There is no net removal of metal if the primary and secondary sludges containing the metals are not permanently removed from the line of flow. For instance, an extended aeration plant passes all the metal to the receiving stream unless secondary sludge is removed.

The copper, chromium, and nickel discharged with the final effluent from an activated sludge plant receiving these metals are predominantly in a soluble form. At an influent concentration of 10 mg/l, only a small amount of zinc is discharged, and this is an insoluble zinc. At higher influent concentrations greater amounts of zinc were discharged as soluble zinc.

EFFECTS OF METALS ON AEROBIC PROCESS

Many investigators of metal toxicity have employed batch operation or direct dosing of the metal to the aeration chamber. Data from individual studies (Table 9) show that primary settling has two

TABLE 8. EFFECTS OF METALS ON MIXED LIQUOR SOLIDS

	Mixed liquor from			
	Control	Metal mixture		
Analysis	unit	8.9 mg/l	4.9 mg/l	2.2 mg/l
Sludge density index	1.5	3.2	3.4	2.4
% Volatile solids	66.7	57.9	61.8	63.8

effects on the metals before entry into the aeration tank. First, the total metal content of the primary effluent is less than that of the influent because some metal is removed with the primary sludge. Second, the chemical and physical characteristics of the wastewater alter the form of the soluble metal introduced. This was especially true in the case of zinc where 90 percent of the added soluble zinc was converted to an insoluble form. The differentiation between soluble and insoluble metal in all studies was made by filtration of the sample through an 0.45 μm membrane filter and by acid digestion of the filtrate before analysis.

The procedure used to determine the concentration of metal in the influent wastewater that would give a barely detectable reduction in efficiency during the aeration phase of treatment is indicated by Figure 20, which shows the results of a study of copper.

During each run, data from an experimental pilot plant unit and a control unit receiving no metal were compared. The metal was added continuously to a constant wastewater feed of the pilot plant unit. Two weeks of acclimation were allowed before data on the quality of the final effluent were collected. This time interval is also required for the metal content of the activated sludge to build up to a condition of operating equilibrium. Final effluents from both units were assayed daily for BOD, COD, suspended solids, and turbidity. The run for any selected metal dosage was continued for 60 days to obtain sufficient data. The values for the two units were then compared as frequency distribution curves. The parameter of effluent quality in Figure 20 is COD; this is plotted as a frequency distribution on arithmetic paper. As shown in the figure, copper present continuously at 0.4 mg/l did not noticeably increase the effluent COD of the experimental unit. A copper concentration of 1.2 mg/l however, caused a significant increase in effluent COD. From this and the other parameters measured, it is concluded that copper present continuously at 1 mg/l in the influent is the threshold dose for the aeration phase.

TABLE 9. METALS IN PRIMARY EFFLUENTS

Metal	Soluble metal introduced in wastewater feed, mg/l	Metal in primary effluent, mg/l	
		Total	Soluble
Chromium (VI)	50	47	38
Copper	10	9	3.0
Nickel	2.5	2.0	1.0
Zinc	10	9	0.6

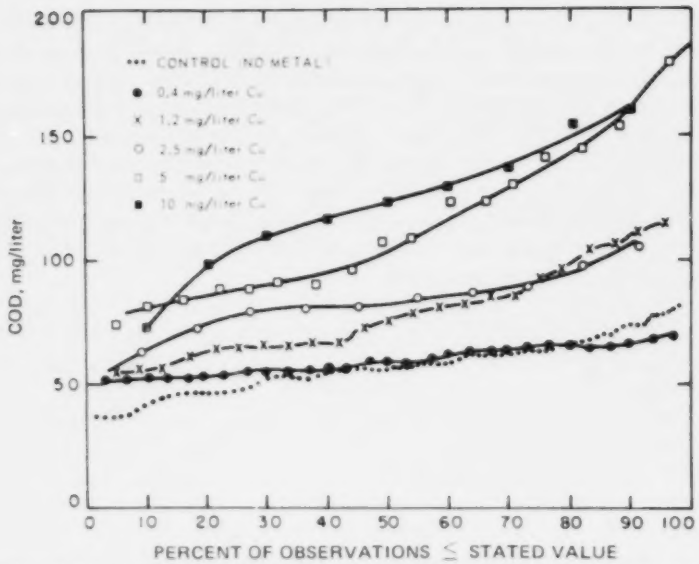


Figure 20. Effect of copper, fed continuously as copper cyanide complex, on COD of final effluents.

It is also useful to plot frequency distribution curves on probability paper. Readily available statistical measurements are given by this type of presentation. If a straight line is obtained with arithmetic probability paper, normal distribution of data is verified. The 50 percent point is very close to the true arithmetic mean of the observations, and the slope of the line is a measure of the standard deviation. Figure 21 is such a plot of data collected during a study of the effects of a mixture of four metals on the activated sludge process. The need for extensive sampling is shown here. The control unit had an average final effluent COD of 45 mg/l; however, continuation of this point to the experimental unit shows that 12 percent of the time the experimental unit's final effluent had a COD of 45 mg/l or less.

Copper and zinc are frequently used by the plating industry as cyanide complexes. These two metals were studied in both the soluble cation form (as sulfate) and as soluble cyanide complexes. Results show that once the activated sludge acclimates to the continuous presence of either form of the metal, there is no difference in effects on treatment efficiency. Figure 22A shows that where turbidity of the final effluent was used as the measure of treatment efficiency, after eight days the system receiving a 10 mg/l

concentration of zinc as cyanide complex had acclimated to cyanide and was producing effluent of stable turbidity. The cyanide content of the effluent followed a similar pattern, with almost complete removal of cyanide at the end of seven days. Figure 22B, from a run with a 20 mg/l concentration of zinc as the sulfate, showed no such acclimation. Direct comparison of the 10 mg/l concentration of zinc sulfate and a 10 mg/l concentration of zinc cyanide complex versus the same control unit is shown in Figure 23. The BOD data collected after two weeks of acclimation, showed no significant difference between the two forms of zinc fed.

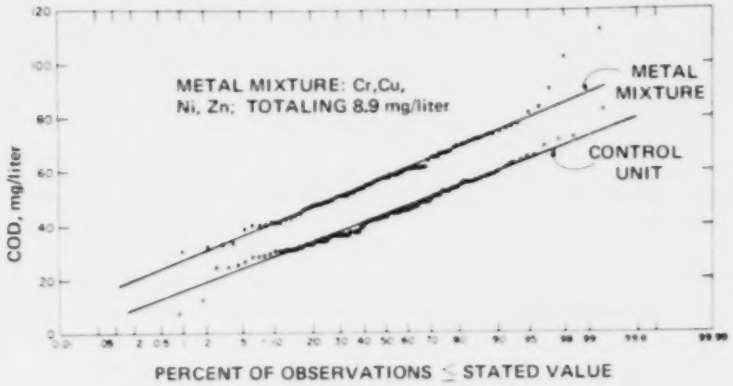


Figure 21. COD of final effluents.

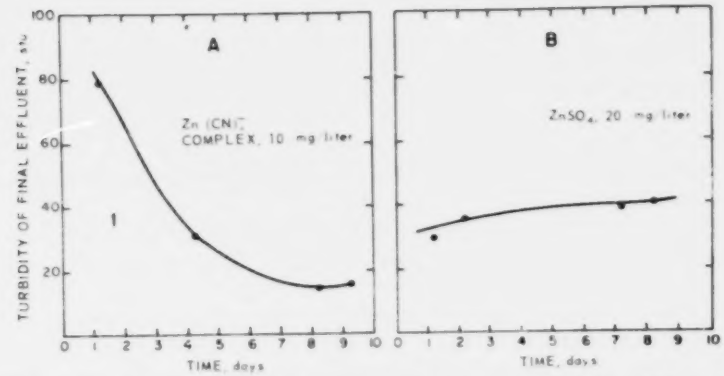


Figure 22. Comparison of acclimation to complexed zinc (A) and zinc sulfate (B).

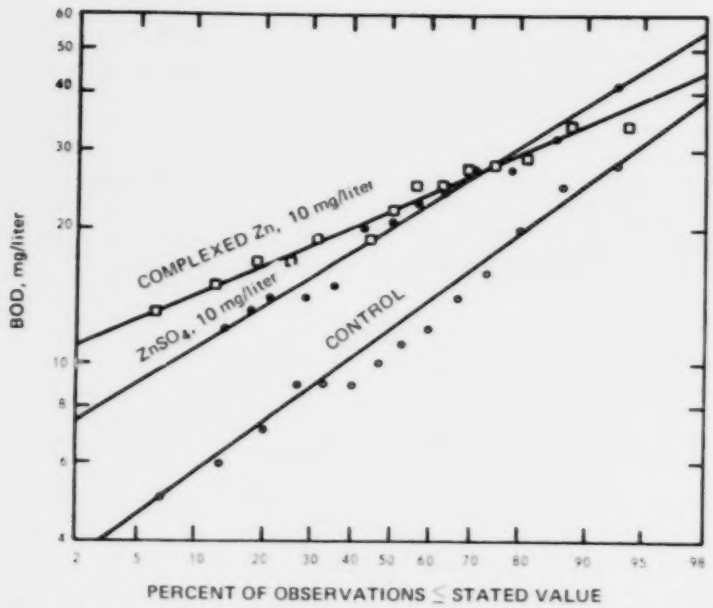


Figure 23. Cumulative frequency data on quality of final effluents with zinc concentration of 10 mg/l in wastewater feed.

The reaction pattern of the activated sludge process was the same for each of the metals studied. A small dose of metal gives a significant reduction in treatment efficiency, but substantially larger doses do not further decrease the efficiency greatly. Figure 24 graphically illustrates this situation. One to two milligrams per liter yield a detectable response after which a plateau is reached, and complete failure does not occur until one to two orders of magnitude are reached.

Table 10 lists the concentrations of metals that give a significant increase in the usual parameters applied to judging treatment efficiency. These may be considered threshold concentrations. It should be borne in mind, however, that these limits were obtained under carefully controlled laboratory operation. The significance of Figure 24 is that the threshold concentration is mainly of academic, rather than practical interest and actual plant situations are concerned with the plateau region of metal dosage and response.

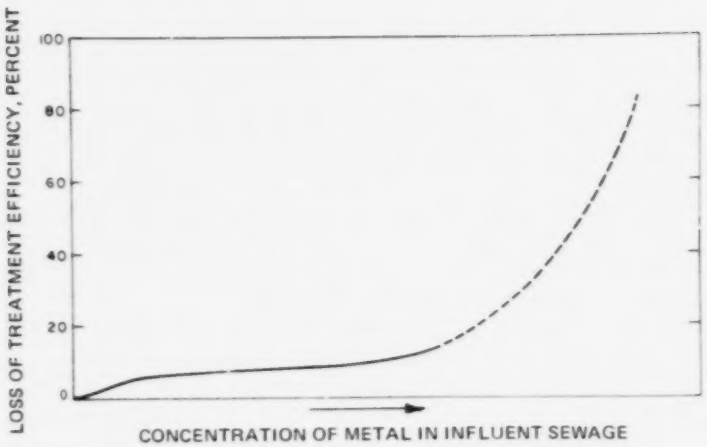


Figure 24. Response of system to metal dosage.

TABLE 10. CONTINUOUS DOSE OF METAL THAT WILL GIVE SIGNIFICANT REDUCTION IN AEROBIC TREATMENT EFFICIENCY

Metal	Concentration in influent wastewater, mg/l
Chromium (VI)	10
Copper	1
Nickel	1–2.5
Zinc	5–10

The results of these studies showed that the aeration phase of biological treatment can tolerate in the influent wastewater, chromium, copper, nickel, and zinc up to a total heavy metal concentration of 10 mg/l, either singly or in combination, with about a 5 percent reduction in overall plant efficiency.

Slug doses of metals to the activated sludge process were also studied. The concentration of metal that constitutes a harmful slug dose is determined by the waste volume, the volume and characteristics of the dilution water, the specific form of the metal, and the usage of the stream below the point of effluent discharge. For convenience, only a single measure of effluent quality, such as an increase in organic material passing through the plant, has been used to

judge a harmful slug dose. As an example, in Figure 21, the control unit has a COD of 70 mg/l or less 98 percent of the time; then a harmful slug dose can be defined as that concentration of metal that will yield an effluent COD in excess of this value for the subsequent 24 hours of performance. The effects of slug doses were observed on 4-hr metal doses to the influent wastewater.

Table 11 gives the results obtained. To fix these concentrations more accurately would require an inordinate amount of time and money. Table 11, therefore, is the best estimate of what concentration of metals causes an exceptional displacement of treatment plant performance as the result of a slug dose.

Not reported in the table are the results of slug studies in which the metals were added as cyanide complexes. In these cases, the cyanide toxicity completely obscured the toxic effect of the metal. In general, acclimation of the system to low concentrations of metals or cyanide did not offer protection from slug doses.

The inhibition of nitrification by heavy metals has been previously studied with regard to individual metals. A pilot plant that received a combination of four metals also showed inhibition of nitrification. There was no evidence of acclimation of the nitrifying organisms to the metals. The oxygen requirement of this metal-loaded sludge was less than that of the control unit because oxygen for the biological transformation of ammonia to nitrate was not utilized. Figure 25 shows the nitrate content of the final effluents of a control and metal-fed unit. Inhibition of nitrification is regarded as an important effect of metal toxicity. A plant so affected would discharge all the influent nitrogen in excess of that needed for synthesis, predominantly in the form of ammonia. Such an effluent would require considerable chlorine if downstream breakpoint chlorination were used, and nitrification in the receiving stream would use large amounts of oxygen.

TABLE 11. METAL CONCENTRATION IN 4-HR SLUG DOSE THAT WILL PRODUCE HARMFUL SLUG, AS MEASURED BY COD

Metal	Concentration influent wastewater, mg/l
Chromium (VI)	>500
Copper	75
Nickel	>50<200
Zinc	160

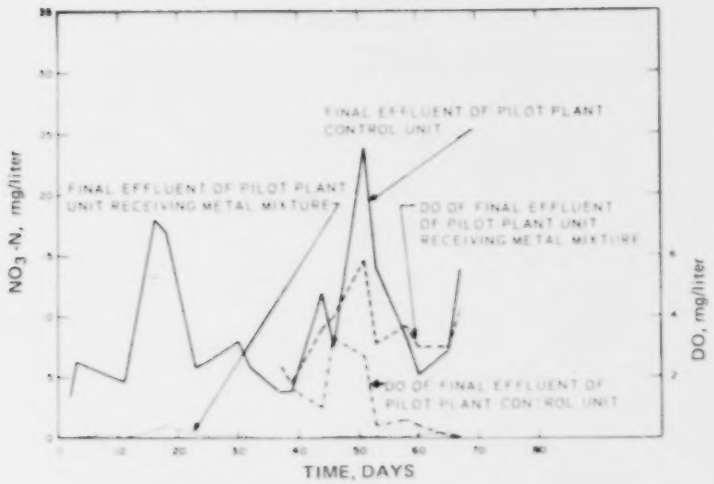

FINAL EFFLUENT OF PILOT PLANT CONTROL UNIT
FINAL EFFLUENT OF PILOT PLANT UNIT RECEIVING METAL MIXTURE
DO OF FINAL EFFLUENT OF PILOT PLANT UNIT RECEIVING METAL MIXTURE
DO OF FINAL EFFLUENT OF PILOT PLANT CONTROL UNIT
NO_3-N, mg/liter
DO, mg/liter
TIME, DAYS

Figure 25. Nitrate nitrogen in final effluents.

EFFECTS ON ANAEROBIC DIGESTION

The metal-bearing sludges produced by the pilot plants were digested in single stage non-mixed digesters. Organic loading was for non-mixed operation. A small circulating pump was used once each day to obtain representative samples of sludge for material balances. In each metal study both primary sludge and combined primary and secondary sludges were digested. The metal contents of the sludges fed to the digesters during several of the runs are given in Table 12. The primary sludges were about 2 percent solids, and the secondary sludges, about 0.5 percent solids during these studies. On a percentage-of-solids basis, the metals in the secondary sludge are concentrated to a much greater extent than in the primary sludge.

A digester receiving combined sludges will contain more metal on a percentage-of-solids basis than a digester receiving primary sludge, when operated at the same influent metal concentration. Digester failure as a result of heavy metals occurs at a lower influent metal concentration in a combined sludge digester than in a primary sludge digester. The maximum continuous influent sewage metal concentrations for satisfactory anaerobic digestion are given in Table 13.

TABLE 12. METAL CONTENT OF SLUDGES FED TO DIGESTERS

Metal	Continuous dose in influent wastewater mg/l	Primary sludge mg/l	Excess activated sludge mg/l
Chromium (VI)	50	330	530
Copper	10	280	160
Nickel	10	62	89
Zinc	10	375	328

TABLE 13. HIGHEST DOSE OF METAL IN CONTINUOUS DOSAGE THAT WILL ALLOW SATISFACTORY ANAEROBIC DIGESTION OF SLUDGES

Metal	Concentrations in influent wastewater, mg/l	
	Primary sludge digestion	Combined sludge digestion
Chromium (VI)	>50	>50[a]
Copper	10	5
Nickel	>40	>10[a]
Zinc	10	10

[a] Higher dose not studied

The response of the anaerobic system to metal dosage does not exhibit a plateau region as does the aeration phase; it is an all-or-none reaction. Digestion either proceeds normally or ceases entirely. This may be more apparent than real, however, because the analytical measures used to assess digester performance are not as direct as those for the aeration phase.

The results of these metal studies show that in the cases of chromium, nickel, and zinc an influent metal concentration of 10 mg/l, either singly or combined, will not affect digestion. Copper continuously present at 10 mg/l causes failure of combined sludge digestion.

The prevailing conditions of anaerobic digestion are such that soluble metal introduced with the feed sludges is efficiently converted to an insoluble form. This is shown in Table 14.

A few slug doses to anaerobic digesters were studied. The slugs to the digesters were in conjunction with the aeration slugs. The sludges produced by the activated sludge process during a metal slug were collected and fed to satisfactorily operating digesters. In no case was there any interruption of digestion caused by the metal-bearing sludges. Concentrations of metals in the influent during these slug studies are given in Table 15.

More detailed studies were not conducted because the logistics of digester operation make it unlikely that an operating digester would be upset by the sludges produced during a slug period. This belief is based on the facts that a digester is not on the main flow stream and only a small part of the total flow through the plant reaches it, and the daily additions to a digester are only a fraction of the total digester volume.

TABLE 14. SOLUBLE METAL CONTENT OF SLUDGES COMPARED WITH TOTAL METAL CONTENT OF DIGESTED SLUDGE

		Soluble metal			Total metal
		Feed sludges			
Metal	Concentration in influent wastewater, mg/l	Primary, mg/l	Excess activated, mg/l	Digested combined, mg/l	Digested combined, mg/l
Chromium (VI)	50	38	32	3	420
Copper	10	2	0.5	0.7	196
Nickel	10	10	9	1.6	70
Zinc	10	0.3	0.1	0.1	341

TABLE 15. DIGESTERS FED COMBINED SLUDGES PRODUCED DURING METAL SLUG TO ACTIVATED SLUDGE PLANT

Metal	Concentration of metal in wastewater feed, mg/l	Effect on digestion
Chromium (VI)	500	None
Copper	410	None
Nickel	200	None

DISCUSSION

The results of these studies show that for each phase of treatment, aerobic or anaerobic, there are different bases for judging the concentrations of metals that are acceptable in the influent wastewater.

The plateau-type response of the aeration phase shows that concentrations of metal many times higher than the threshold concentration can be received without greatly reducing efficiency. In a situation in which removal of organic matter is not critical, the most sensitive performance criterion may be the ability of the digester to handle the sludge produced. Generally, biological secondary treatment processes can tolerate up to 5 mg/l of the inorganic toxicants without noticeable impairment of treatment efficiency. The composition of municipal wastewater and the chemistry of the inorganic toxics is such that disproportion of the inorganics occurs during treatment and the materials are conservative in nature. Thus, if the inorganics enter the treatment facilities at concentrations of 1 to 5 mg/l in the raw water and removal occurs in the system, to yield low effluent residuals, the inorganics will be found in concentrated side streams such as primary sludge, waste biological sludge, digested sludge, digester supernatant, or lagoon bottom sediment. Mainly, the inorganics will exist as insoluble products in these side streams or sludge deposits. Assessment of technology to enhance inorganic toxics control must be based on the overall environmental trade-off of low final effluent residuals versus concentration of the inorganics in the sludge or sludge handling operations of municipal treatment systems and their subsequent fate during ultimate disposal practices.

In other cases the amounts of metals passing through the plant to the receiving stream may be the factor that determines the concentration of metals permissible in the plant influent. Metals are not destroyed in wastewater treatment processes, so management practices must be based on their distribution in various process streams.

A survey of four municipal treatment plants (Ref. 18, Chap. 10) concerning the receipt of heavy metals, distribution of metals in the process outlets, and effects of metals on treatment efficiency, has shown a pattern of response similar to pilot plant studies.

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